ECOLOGY OF SMUT DISEASES IN GRASSES: INCIDENCE AND EFFECT IN NATURAL POPULATIONS

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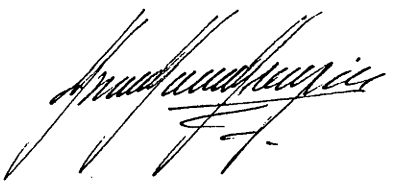
DECLARATION

The research presented in this thesis is my original and independent work. Specific contributions and assistance by others are referred to in the text and acknowledgments.



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ABSTRACT

Fungal pathogens play an important role in shaping the size and structure of plant populations by killing or reducing the fecundity of individual plants. The diversity of fungal pathogens is very great and their interaction with hosts is clearly affected by a broad amalgam of factors, not least of which are traits related to the life-histories of both host and pathogen. One such major life-history difference is whether pathogens are systemic or non-systemic. It has been suggested that this factor alone may be responsible for major differences in host growth form and may be of importance in explaining the outcome of systemic infections. However to date, the study of the effects of systemic diseases in natural systems has been restricted to associations involving dicotyledonous plants. Thus the purpose of this thesis was to extend this work to include monocotyledonous plants. In particular I aimed to study the effects of three species of systemic flower-infecting smut fungi on the performance of their grass hosts, and to interrelate this with aspects of regional and local variation in disease incidence. The host-pathogen systems studied here vary in their growth form: *Ustilago bullata* infecting the tiller-forming grass *Bromus catharticus*; *Ustilago cynodontis* infecting the clonal grass *Cynodon dactylon*; and *Sporisorium amphilophis* infecting the tiller-forming grass *Bothriochloa macra.*

Results from a series of glasshouse-based experiments showed that smut infection had a range of differential effects on the three host grasses, some of which reflect their growth form. In all three associations, infection resulted in the complete sterility of host plants, reduced growth and changes in the allocation of resources from root to shoots. Survival and the competitive ability of infected plants was generally unaffected, but the effects of smut infection on these parameters were dependent on the environmental conditions under which the plants were growing. Smut disease did not affect survival of *Bromus catharticus* and *Bothriochloa macra* plants. In normal potting mix infection reduced the size of plants but **not** the competitive ability. In these circumstances healthy and infected plants competed equally for the same limiting resources. However, under the environmental stress of low nutrient conditions infection reduced the competitive ability of infected plants. These results are quite different to those

previously found in non-systemic systems. There was no escape from disease in these systems. In contrast, in the *Cynodon dactylon -Ustilago cynodontis* system mortality of infected plants was higher when grown at high nutrient levels, but the competitive ability of infected plants was not affected by smut, even when grown in poor environments. Disease spread within the stolons of *C. dactylon* was found to be incomplete. Smut infection did not have any significant effect on the germination of smut infected *B. catharticus* or *C. dactylon* seeds.

Regional variation in the incidence of the systemic floral-smut fungus *Sporisorium amphilophis* on the perennial grass *Bothriochloa macra* was investigated through three surveys over a 12-year period (1981-1993). In all three surveys a marked north-south trend in percentage of infection was detected with a greater proportion of plants in northerly populations being infected than those in populations located to the south. A negative relationship between the incidence of disease in populations and low temperature was found. Detailed exploration of local variation in a subset of five populations, showed that the incidence of *S. amphilophis* was density-dependent and that there was a noticeable spatial patterning in the distribution of infected plants. Disease, which prevents seed production and negatively affects different aspects of the morphology of the plants, such as the height and basal diameter, incidence was greater in the edge areas of the host populations that were subject to more disturbance than were the core areas. These field results were complemented by glasshouse-based competition experiments, the results of which helped explain the distribution of diseased plants within individual populations. Further experiments investigated the process of infection and the role of phalacrid beetles in this host-pathogen interaction.

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CHAPTER 1

General Introduction

Most studies involving pathogens and plants have been in agricultural systems, where pathogens are frequently the cause of substantial yield reduction or total loss of crops. Evidence from these systems, indicates that pathogens play an important role in the population dynamics of very many plant species of agronomic interest (Agrios 1978). In contrast, the study of the interactions of pathogens with their host plants under natural conditions has been very much neglected. Indeed, it has been argued that the ecological and genetic simplicity of agricultural systems (extensive monocultures of genetically uniform cultivars) is the cause of their disease vulnerability while epidemics of pathogens rarely occur in natural systems (Dinoor and Eshed 1984), and therefore their interactions with their hosts have been seen more as a kind of benign association (Browning 1974). However, interest in plant-pathogen interactions in natural communities has increased and a number of studies (Alexander 1990a; Augspurger and Kelly 1984; Jarosz and Burdon 1992) have now shown that epidemics are not uncommon (Burdon, 1993) and that fungal pathogens play an important role affecting the fitness of natural populations by killing or reducing fecundity of host plants (Burdon, 1991).

The effect that plant pathogens have on individual host fitness is greatly affected by the phase of the host's life cycle when infection occurs (Burdon, 1987). Germinating seeds and young seedlings of many species of plants, for example are susceptible to attack by soil-borne pathogens that can cause a range of 'damping-off' diseases. Infection at these stages of the life cycle of a plant often results in rapid death and, therefore, in the total loss of individual fitness. Moreover, by differentially killing greater populations of individuals in high density stands than low density ones, or of plants growing in microsites particularly favourable to the pathogen, such pathogens can also affect the distribution of plants, such as seedlings of various species of tropical trees (Augspurger 1983,1984; Augspurger and Kelly 1984).

Adult plants are also affected in many different ways and by a great variety of pathogens. Infection in the adult stages can result in reduced survival of plants (eg. *Senecio vulgaris* infected by the rust *Puccinia lagenophorae*; Paul and Ayres, 1986d and *Melampsora lini* infecting *Linum marginale*; Jarosz and Burdon 1992), or seed production. Such reductions in fecundity may result from the effects of pathogens that directly attack floral organs or developing embryos. For example, the systemic smut *Microbotryum violaceum* causes the complete sterilisation of many species of the family Caryophyllaceae, by causing the abortion of female reproductive structures and replacing the anthers of infected plants with fungal teliospores (Alexander and Maltby 1990). For many pathogens, however, effects on fecundity, growth (eg. *Erysiphe graminis hordei* infecting barley; Brooks 1972), and intraspecific competition (eg. *Puccinia chondrillina* infecting *Chondrilla juncea*; Burdon *et al.* 1981) may result from the cumulative effects of foliar lesions. Then effects may cascade from those acting on the individual to influence the genetic structure of host plant populations (Burdon and Jarosz, 1988) and even the distribution of plant species. An interesting example that describe the potential importance of pathogens in the structure of plant communities, is found in foredune vegetation, where natural succession of plant species has been attributed to the occurrence of plant-specific soil-borne diseases (Van der Putten *et al.* 1993).

Despite the great diversity of pathogens, to date most studies have focussed on discrete lesion diseases or those that have obvious and devastating effects, such as rusts, mildews or wilts. In contrast, the study of systemic diseases in natural systems is limited. However, many of these diseases are particularly interesting because they appear to occupy an intermediate position between the highly parasitic effects of many non-systemic pathogens and the symbiotic consequences of some endophytic infections. Thus other than the effects of floral castration (the replacement of floral parts by fungal stroma and spores) with its obvious consequences for the Darwinian fitness of many plants, some systemic infections can enhance the vegetative vigour of infected plants (Wennström and Ericson 1991) or may have no effect on the competitive ability of the infected individuals (Carlsson and Elmqvist 1992), but affect survival under specific environmental conditions for example, harsh winters (Thrall and Jarosz 1994).

Systemic infections, such as those caused by systemic floral smuts and systemic rusts, can spread internally to other parts of the host living intercellularly within the plant tissues. Since these pathogens are perennial it is clearly disadvantageous

for them to kill their hosts immediately. Rather by keeping the host alive, such pathogens can continue to sporulate and spread over a number of seasons - a strategy that seems to be common among systemic pathogens. However, the effect that plant pathogens have on individual host fitness is determined by a variety of factors, such as the phase of the host's life cycle when the infection occurs (Burdon, 1987) and the growth patterns exhibited by the host (Wennström 1994). Some studies suggest that the growth form of the host may be of importance in explaining the outcome of systemic infections. Thus systemic pathogens infecting plants with strong lateral growth generally have more severe effects on their hosts - reducing survival of shoots but not necessarily causing the death of the whole clone - than do pathogens infecting plants with weak lateral growth (Wennström 1994). In the latter situation, where weak lateral growth prevents spatial escape, pathogens seem to have a more subtle effect.

Currently, knowledge of the effects of systemic diseases in natural systems is very largely restricted to those involving dicotyledonous hosts (Alexander and Antonovics 1988; Alexander and Maltby 1990; Wennström and Ericson 1990; Carlsson and Elmqvist 1992; Jennersten *et. al.* 1983). Investigation of interactions between monocotyledonous hosts and systemic diseases, particularly flower infecting smuts have been mainly restricted to annual grasses of agronomic importance. These investigations have shown that infection of grasses by smuts results in a reduction in the vigour and survival of seedlings (Falloon 1976, 1979a; Doling 1964), seed production, vegetative growth, plant survival (Falloon *et al.* 1988), and the ability of infected plants to compete (Doling 1964). However these studies have generally focussed on the effect of pathogens on the performance of plants on an area basis rather than on the effects of pathogens on the Darwinian fitness of hosts. As a consequence, with the exception of Kirby's survey of the floral smut *Ustilago spinificis* incidence in populations of *Spinifex* (Kirby 1988), the incidence of smut diseases in natural populations and their effect on the biology of their hosts is not well known.

A central issue for the understanding of host-pathogen dynamics in these systems, is to determine the response of systemic pathogens to hosts with different life-histories. This is likely to be a critical factor in determining the short-term outcome of interactions and their evolutionary consequences. Because of previous neglect, in this thesis I have focussed on the effects of systemic flower-infecting smuts on three monocotyledonous species which show a range of life strategies. In particular, the broad aims of my work were to study the effects of three species of

systemic flower-infecting smut fungi on the performance of their specific host grasses and, in the case of one interaction (the flower-infecting systemic smut *Sporisorium amphilophis* and the grass *Bothriochloa macra*), to interrelate this with aspects of regional and local variation in disease incidence. To achieve these aims a series of glasshouse experiments and field surveys have been employed.

The three smut-host systems analyzed were:

(a) *Ustilago bullata* infecting the short-lived perennial, tillering grass *Bromus catharticus*,
(b) *Ustilago cynodontis* infecting the perennial clonal grass *Cynodon dactylon*, and
(c) *Sporisorium amphilophis* infecting the perennial native tillering grass *Bothriochloa macra.*

I use variation in plant density, soil nutrient levels and the relative frequency of infected and healthy plants in stands to provide a range of biotic and abiotic conditions against which to determine:

(i) the effects of smut infection on growth and survival of *Bromus catharticus*, *Cynodon dactylon* and *Bothriochloa macra*;
(ii) how infection affects the competitive ability of the host grass and allocation of resources within infected plants;
(iii) how infection affects germination and establishment of seedlings of *Bromus catharticus* and *Cynodon dactylon*; and
(iv) the importance of host growth pattern on disease expression in *C. dactylon.*

The effects of smut infection on the performance of the three grass species were compared in order to determine patterns that could explain how the dynamics of plant populations and communities can be affected by systemic pathogens.

To study aspects of regional and local variation in the incidence of the flower-infecting systemic smut *Sporisorium amphilophis* in natural populations of the grass *Bothriochloa macra* I addressed the following specific aims:

(i) to assess regional variation in the levels of infection;

(ii) to determine spatial patterns of occurrence of disease in relation to host density, and spatial heterogeneity of the plant populations;

(iii) to determine the competitive ability of infected and healthy plants growing under controlled conditions;

(iv) to study the role played by insects, particularly phalacrid beetles, in the spread of the smut disease in natural populations of *B. macra.*

Outline of chapters

This thesis has been divided into three sections. In Section A I have included two chapters related to the effects of smut infection on *Bromus catharticus* and the clonal grass *Cynodon dactylon.* Section B includes three chapters related to the association between *Sporisorium amphilophis* and its host *Bothriochloa macra..* Section C includes a general discussion of the results presented in the preceding chapters. Each chapter in these sections has been written as an individual manuscript that stands alone. This necessarily has resulted in a certain degree of repetition. A brief description of each chapter follows:

Chapter 1. General introduction.

SECTION A

Chapter 2. Explores the effects of the systemic smut *Ustilago bullata* infecting *Bromus catharticus* on the competitive ability of host plants, the allocation of resources within infected plants as well as the possible differential effects of infection at different stages of the host life-cycle.

Chapter 3. Explores the effect of the systemic flower smut *Ustilago cynodontis* on the establishment, growth, survival and competitiveness of the perennial clonal grass *Cynodon dactylon.*

SECTION B

Chapter 4. Examines the ecology of a Australian native systemic interaction between the flower-infecting smut *Sporisorium amphilophis* and the grass *Bothriochloa macra.* In particular this chapter explores the regional (among

populations) and local (within populations) variation in disease incidence as well as the effects of smut infection on growth, survival and competitiveness of its host plant.

Chapter 5. Investigates possible mechanisms of infection of *Bothriochloa macra* by the systemic flower smut *Sporisorium amphilophis.*

Chapter 6. Explores the possible role of a Phalacrid beetle, in the dynamics of smut infection on *Bothriochloa macra.*

SECTION C

Chapter 7. General discussion of the results found in the preceding chapters.

SECTION A

EFFECTS OF SMUT INFECTION

This section explores the effects of systemic smut infection on *Bromus catharticus* and the clonal grass *Cynodon dactylon*. Chapter 2 describes the effects of the flower-smut *Ustilago bullata* on the competitive ability of *Bromus catharticus* and the possible differential effects of infection at different stages of the host life-cycle. Chapter 3 focuses on the effects of the flower-smut fungus *Ustilago cynodontis* on the establishment, growth, survival and competitiveness of the perennial clonal grass *Cynodon dactylon*.

CHAPTER 2

Effects of the systemic flower infecting-smut *Ustilago bullata* Berk. on the grass *Bromus catharticus* J. Vahl.

INTRODUCTION

Host-pathogen interactions are typified by a wide variety of effects that have a diversity of consequences for the Darwinian fitness of both hosts and pathogens. Plant pathogens may kill their hosts rapidly (eg. damping-off diseases); cause individually small, but cumulatively debilitating effects on fecundity or longevity (eg. mildews); or may castrate their hosts reducing fecundity to near zero while having less effect on longevity (eg. systemic floral smuts; Burdon 1993). Inevitably, these very different styles of parasitism impose quite different selective forces on pathogens. These styles of parasitism may conveniently be seen as a continuum ranging from extreme ***r*** types characterized by high fecundity and short life-cycles (many rusts and mildews); through ***K*** types characterized by lower fecundity, longer life-cycles and intimate associations with their hosts (systemic floral smuts and systemic rusts), to finally, the ultimate expression of the mutualistic associations developed by endophytic fungi in which the 'pathogen' is transmitted transovarially (Clay 1991). At the extremes of this continuum, selective forces appear to favour quite different evolutionary outcomes - high aggressivity in pathogens at the ***r*** end of the spectrum; much more subtle, benign interactions at the ***K*** end.

The interactions involving pathogens that induce systemic diseases of their hosts potentially combine selective elements from both ends of the ***r-K*** continuum. Such interactions are typified by pathogens that form perennial infections that spread systemically throughout their hosts. Survival of these infections inevitably depends on the survival of their host individuals. However, efficient spread of the pathogen to uninfected host individuals depends on the production and dissemination of spores. These different needs would seem to favour both ***K*** and ***r*** selection simultaneously, thus make these interactions particularly interesting ones to study.

A first step in understanding the long-term evolutionary consequences of these conflicting forces can be achieved by examining the effects of systemic pathogens on host growth, fecundity and competitive ability. To date virtually all studies that take such an ecological approach have focused on perennial, dicotyledonous plants. In these, the consequences of infection have been variable. Initial studies of the interaction between *Microbotryum violaceum* and *Silene alba* failed to detect any significant difference in the growth and survival of healthy and infected plants (Alexander and Antonovics 1988). More recently though, field work on the same system has shown that infected plants suffer greater mortality than healthy ones during hard winters (Thrall and Jarosz 1994). For other associations such as *Puccinia punctiformis* infecting *Cirsium arvense*, the impact of rust infection depends on factors such as site characteristics and type of infection (systemic or local; Frantzen 1994). In contrast, increased vigour of infected plants has been observed in other systems such as *Pulsatilla pratensis* infected by *Puccinia pulsatillae* (Wennström and Ericson 1991). However, in none of these examples has it been possible to dissect out the effect of the disease over the full life cycle of host plants.

Systemic smut diseases are also common pathogens of many grass species. These interactions have major advantages as experimental tools as the hosts are frequently annual or very short-lived perennials and have a wide range of different growth forms that can be used to increase our understanding of the interplay of host growth-form and systemic pathogen spread and persistence (Wennström and Ericson 1992). Despite this, work on them is very limited. In this chapter I analyze the effects of the floral-smut disease *Ustilago bullata* Berk. on the perennial grass *Bromus catharticus* J. Vahl. Specifically I use variations in plant density, soil nutrient levels and the relative frequency of infected and healthy plants in stands to provide a range of biotic and abiotic conditions against which to determine: (i) does the effects of infection by *U. bullata* extend beyond a simple reduction of seed production?; (ii) how infection affects the competitive ability of *B. catharticus* and allocation of resources within infected plants; and (iii) at what stages in the life cycle these effects are most apparent.

METHODS

Host-pathogen association

Bromus catharticus J. Vahl is a densely tufted, short-lived perennial grass native to South America. Following its introduction to Australia as a pasture plant it has become a widespread weed growing on disturbed ground and along roadsides (Lamp *et al.* 1990). In these situations, *B. catharticus* is frequently affected by the systemic floral smut fungus *Ustilago bullata* Berk. The presence of the smut fungus in its host becomes apparent at anthesis when the glumes and ovary of infected hosts are destroyed being replaced by a dark brown-black mass of teliospores (Falloon *et al.* 1988).

Infection of host plants by *U. bullata* may occur through either: (i) penetration of the coleoptile of germinating seed (seedling infection); or (ii) direct infection of young tillers (shoot infection). Of the two methods seedling infection is the most common with pathogen spores being carried on the outside of the seed or occurring in the soil. Once infection occurs the mycelium grows throughout the tissues of the host and remains systemic within the crown region throughout the vegetative life of the plant. As the inflorescences develop the spores develop in the spikelets. All inflorescences produced are smutted (Falloon *et al.* 1988; Fig. 1).

Previous agronomic studies of this host-pathogen interaction suggested that infection by *U. bullata* could affect the establishment, growth and fecundity of *B. catharticus* (Falloon and Hume 1988). However, that study focussed attention on the effect of the pathogen on performance on an area basis rather than more explicitly questioning its effect on fitness components of plants. Here, I do this by subdividing a study of the effect of the smut on the life cycle of *B. catharticus* (dispersed seed to reproductive adult (I; Fig. 2) into its component parts of: the effect on germination and early establishment (II) ; and the effect on the growth and competitive ability of established plants (III; Fig. 2). These effects were examined at two soil nutrient levels to induce different levels of inter-plant competition.

Fig. 1. The effects of infection by the floral-smut fungus *Ustilago bullata* on *Bromus catharticus* Left: smutted heads; right: healthy inflorescences.

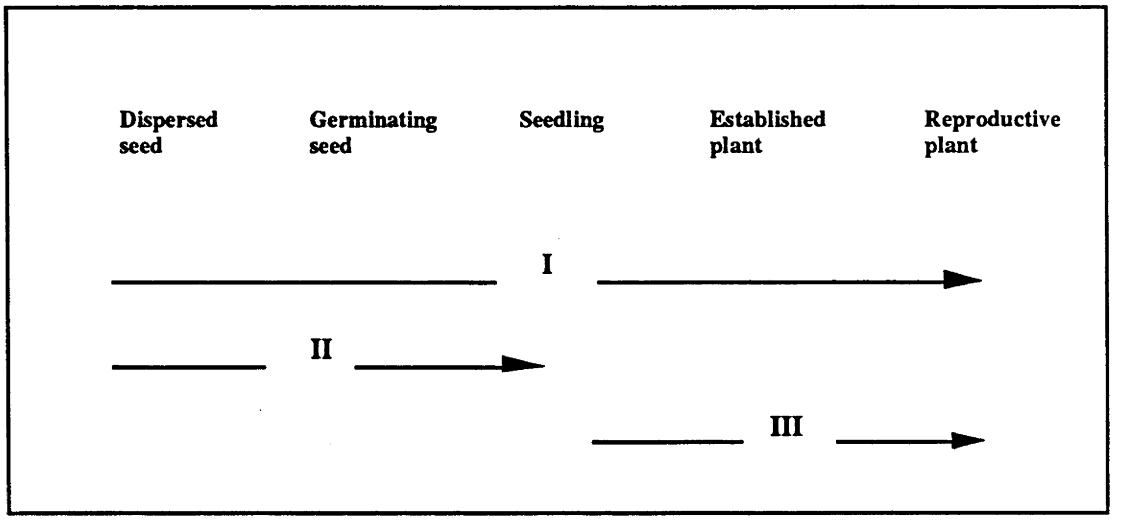


Fig. 2. Diagrammatic representation of the stage of the life cycle of *Bromus catharticus* examined in the three experiments (I, II, III) examining the effects of the pathogen *Ustilago bullata* on host plant fitness.

Effect of *U. bullata* on the competitive ability of *B. catharticus*

The effect of smut disease on the competitive ability of *B. catharticus* was examined in two experiments (I and III, Fig. 2). Experiment I assessed the cumulative effect of smut disease over the entire life cycle of *B. catharticus* plants: that is- the germination of seed, establishment of seedlings and the later effect on the competitiveness of adult plants. In experiment III I aimed to exclude the effect of the smut during the seedling phase and assess only its effects on the performance of plants from established seedlings to flowering.

For both experiments, 0.5g lots of *B. catharticus* seed were vacuum-inoculated in 0.1 g of freshly collected teliospores of *U. bullata* suspended in 25 ml of distilled water. Following this treatment the seed was left in the suspension for a further 24 hrs. A control, uninoculated treatment was provided by soaking 0.5 g of seed in the same volume of sterile distilled water for 24 hours. Hereafter, inoculated plants are referred to as infected and controls as healthy plants. In experiment I the inoculated and control seeds were sown directly in pots following an experimental design involving three planting densities (4, 8 and 16

plants per pot) and five frequencies of infection (0, 25, 50, 75 and 100% of infected plants per pot). In experiment III infected and healthy seeds were planted in separate flats, and kept in a naturally-lit glasshouse until germination and establishment occurred two weeks later. Once established, similar sized infected and healthy seedlings were transplanted into a series of 15 cm diameter pots following an experiment design involving three planting densities (4, 8 and 16 plants per pot) and three frequencies of infection (0, 25, and 100% of infected plants per pot). All pots were filled with potting mix. An additional experiment looking at the effect of nutrient status on the development of this host-pathogen interaction was carried out at the same time. For both experiments I and III the 16 plants per pot treatment was replicated in a lower nutrient soil mix (75% sand: 25% potting mix).

All pots were kept in a randomized array in a naturally-lit glasshouse (temperature 18-24 °C) and watered daily to field capacity. Plants were harvested after 4 months at which time it was possible to confirm by the presence of infected inflorescences that all inoculated plants were indeed infected with *U. bullata*. The roots of harvested plants were washed and all plants dried at 60 °C for one week. Shoot and root weights were recorded separately for each individual and the number of dead plants per pot was scored.

Data analysis: Description of the effects of smut-disease on the competitive performance of *B. catharticus* was achieved by using maximum-likelihood estimates for the parameters of the non-linear de Wit competition model (Machin and Sanderson 1977) to determine values for k (the relative crowding coefficient). Values of k give a quantitative measure of the relative competitiveness of two species: k values greater than unity imply the species concerned is more aggressive than the species with k values less than unity.

The model initially assumes that the two species are not competing for the same resources by fitting the data to the $k_{hd} \neq 1/k_{dh}$ submodel. It then fits the data to the second submodel, that is $k_{hd}=1/k_{dh}$ (the two species are competing for the same resources but are unequal in their relative competitive ability). If the second submodel is not a significantly better fit, then the first , ie. $k_{hd} \neq 1/k_{dh}$ is the model accepted. However, if there is a significant difference, a third submodel is fitted to the data - that $k_{hd}=1/k_{dh}=1$. In this circumstance the two species are competing for the same resources and are equally competitive.

Again if the third submodel is not significantly better, the second submodel is accepted. This sequential process of model fitting progressively reduces the number of parameters estimated as it works from the maximal to the most constrained model.

To determine the effect of density, smut-disease, the proportion of infected individuals and nutrient levels on the root/shoot (R/S) ratios and total dry weights (expt. III) of *B. catharticus* plants, data were analysed using an unbalanced linear mixed model; a restricted maximum likelihood estimation (REML) was required (Engel 1990). Root/shoot ratios were log transformed prior to analysis. Survival was analysed using logistic regression (McCullagh and Nelder 1989). The fitted terms considered were density, proportion plants infected per pot, disease status and nutrient level.

Effect of *Ustilago bullata* on germination and emergence of *Bromus catharticus*

The effect of the smut pathogen on the proportion of seeds germinating and time to emergence of *B. catharticus* seedlings was examined during the summer of 1993 (II, Fig. 2). 0.5 g lots of seed were vacuum-inoculated for 15 minutes in 0.1 g of freshly collected teliospores of *U. bullata* suspended in 25 ml of distilled water. Following this treatment the seed was left in the suspension for a further 24 hrs. A control, uninoculated treatment was provided by soaking 0.5 g of seed in 25 ml of sterile distilled water for 24 hours. Following these treatments, 100 seeds were sown at a standard density, in separate plastic flats filled with potting mix. Each treatment was replicated three times. The flats were kept in a naturally lit glasshouse (18-24 °C) and the time to emergence of each seedling recorded. Seedlings were deemed to have germinated when the plumule was visible above the soil surface.

Data analysis: The effect of smut disease on the germination and establishment was analysed using logistic regression (McCullagh and Nelder 1989). The response variables were the total number of seedlings at the end of the experiment and number of new seedlings per day. Differences in these variables were explained in terms of disease status, concentration of spores and time (days).

RESULTS

Experiment I: Effect of *Ustilago bullata* on the life-cycle long competitive performance of *Bromus catharticus*

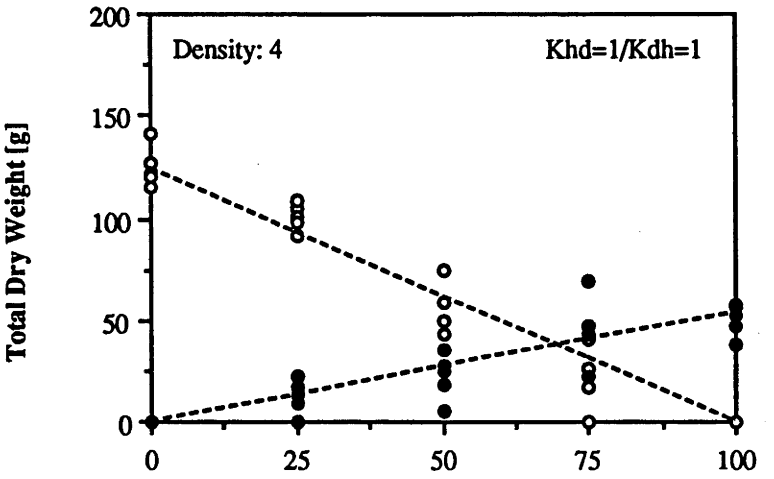
Competitive relationships

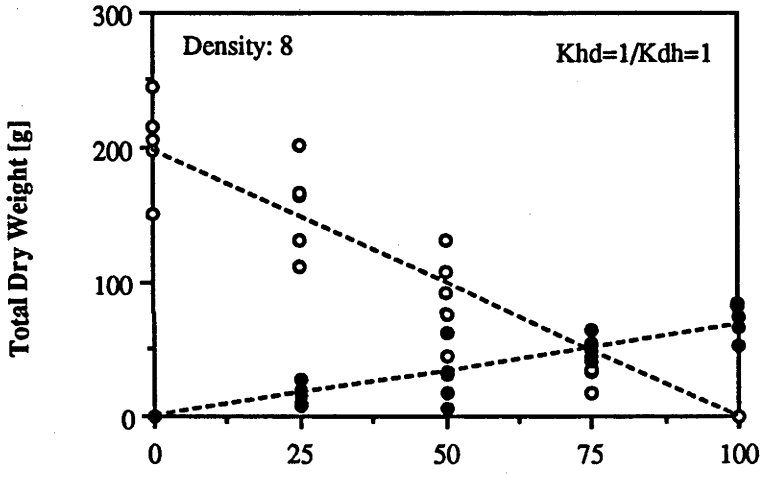
The general effect of *U. bullata* on competition between healthy and infected *B. catharticus* plants grown at different densities and relative proportion in mixture is shown in Fig. 3. Both healthy and infected plants were smaller at higher densities. At all densities, the pathogen substantially depressed the yield of infected individuals over that of healthy plants growing under the same conditions. Surprisingly though, quantitative descriptions of the competitive performance of infected and healthy individuals determined by the relative crowding coefficient (k) indicated that they did not differ significantly from the null model (k_{hd}=1/k_{dh}=1). That is at all three densities, the competitive ability of healthy (k_{hd}) and infected plants (k_{dh}) did not differ. Healthy and infected plants were equally efficient in competing for resources.

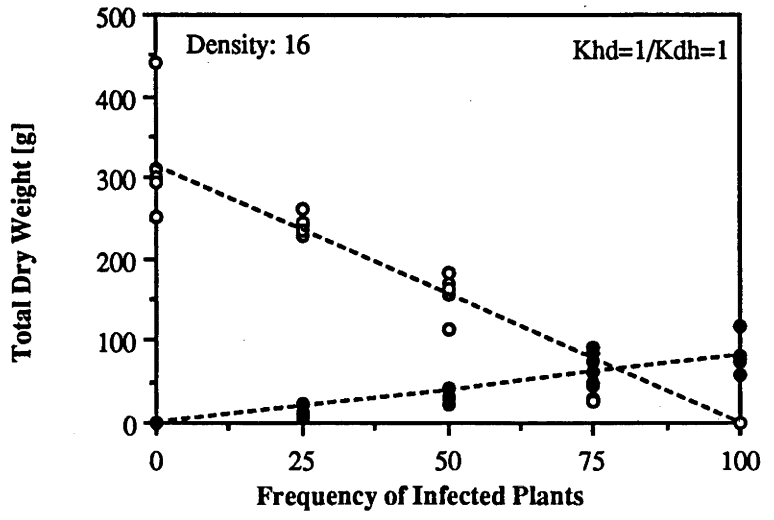
Effect of the level of nutrients on competition

Both healthy and infected plants grown at low nutrient levels were smaller than plants subject to equivalent treatments growing at high nutrient levels. As seen previously at high nutrient levels, crowding coefficient values did not differ significantly from unity, suggesting that healthy and infected plants competed with equally efficiency for resources (K_{hd} = 1 /K_{dh} =1; Fig. 4). In contrast, at low nutrient levels the model providing best fit indicated that while healthy and infected plants competed for the same resources, healthy plants were more aggressive than infected ones (K_{hd} = 1.52, K_{dh} = 0.66; Fig. 4) .

Fig. 3. Replacement series for competition from germination to flowering between healthy and infected *B. catharticus* plants (Expt. I). The relative crowding coefficients of healthy and infected plants (k_{hd} and k_{dh}) are presented. The dashed lines show the fitted relationship $k_{hd} = 1/k_{dh} = 1$; open circles = individual replicate values, healthy plants; closed circles = individual replicate values, infected plants.


Density: 4
Khd=1/Kdh=1
Total Dry Weight [g]
200
150
100
50
0
0
25
50
75
100


Density: 8
Khd=1/Kdh=1
Total Dry Weight [g]
300
200
100
0
0
25
50
75
100


Density: 16
Khd=1/Kdh=1
Total Dry Weight [g]
500
400
300
200
100
0
0
25
50
75
100
Frequency of Infected Plants

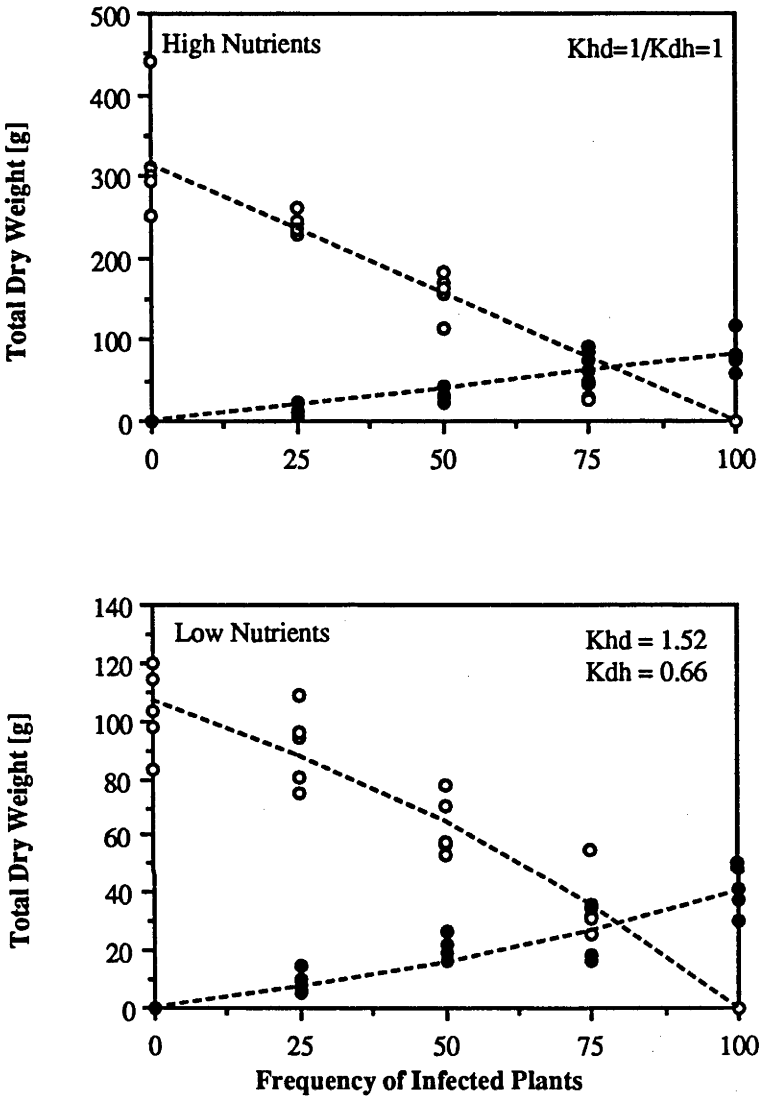


Fig. 4. Replacement series for competition from germination to flowering between healthy and infected *B. catharticus* plants growing at two nutrient levels and a density of16 plants/pot. [The high nutrient results are the same as those shown in Fig. 3]. The relative crowding coefficients (K_{hd} and K_{dh}) are presented. The dashed lines show the fitted relationship $k_{hd} = 1/k_{dh} = 1$ (high nutrients) and $k_{hd} = 1.52$, $k_{dh} = 0.66$ (low nutrients); open circles = individual replicate values, healthy plants; closed circles = individual replicate values, infected plants.

Effects of infection on root / shoot ratio

Infection by *U. bullata* had a substantial effect on the distribution of resources between roots and shoots within individual plants (Fig. 5). Root to shoot ratios were significantly greater in healthy than infected plants and differed between the different density treatments ($P < 0.001$, linear mixed model; Engel 1990). However, neither the frequency of infected plants / pot nor the interaction of this variable with density was significant. While smut infection led to a substantial reduction in the relative allocation of resources to the roots of infected plants, this division of resources remained similar over the entire range of plant densities (Fig. 5).

Soil nutrient levels had a significant effect on resource allocation patterns within healthy and infected plants (Fig. 5). In both the R/S ratio was substantially greater under low nutrient conditions although the change in allocation pattern was more substantial for infected plants (3 - fold increase in R/S ratio) than for healthy ones (2 - fold increase).

Survivorship

There was no significant effect of infection or plant density on the survival of plants growing under high nutrient conditions at the three different densities ($P > 0.05$). However, for both infected and healthy plants, mortality was always greater in mixtures than monocultures and more frequent among infected than among healthy individuals. This general trend was also apparent in the high and low nutrient treatment comparison (density of 16 plants/pot) where the survival of *B. catharticus* plants was generally affected by the pathogen, however differences in survival among healthy and infected plants were not statistically significant ($P > 0.05$).

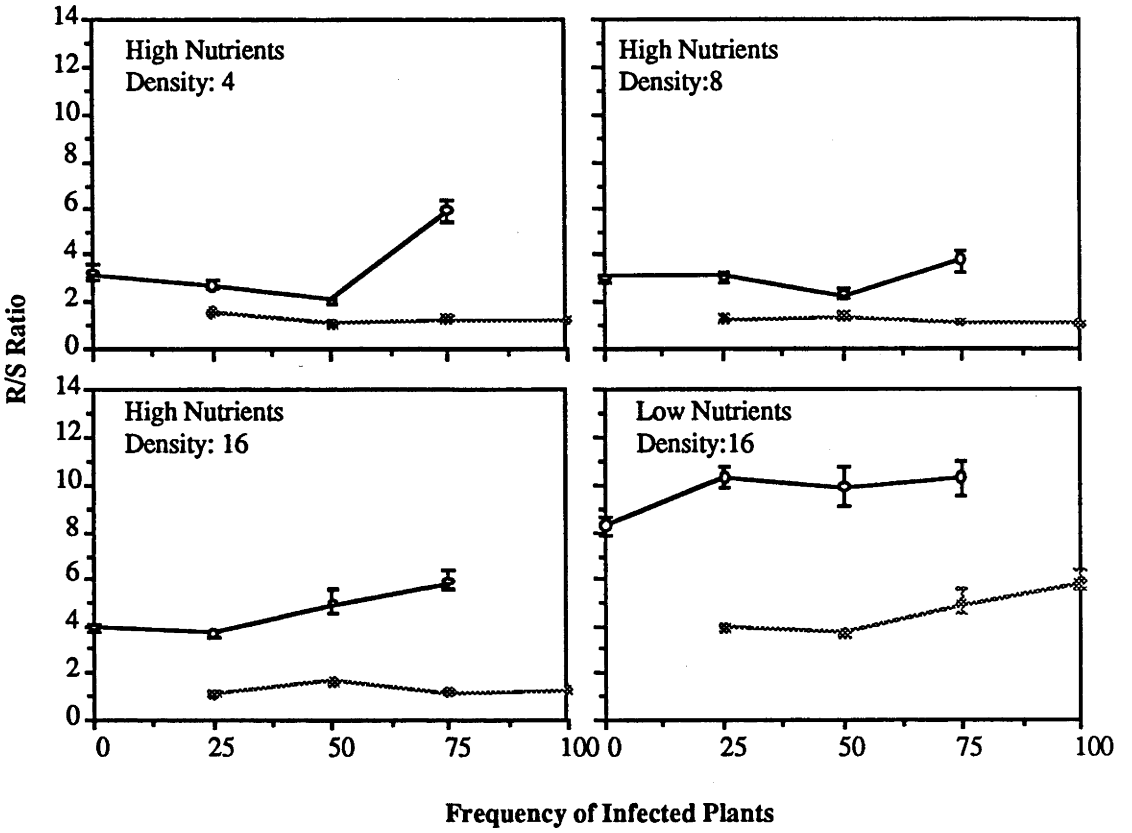


Fig. 5. Resource allocation between roots and shoots of healthy and infected *B. catharticus* plants growing at three densities (4, 8, 16 plants/pot) and two levels of nutrients (high and low). Vertical bars represent ± 1 SE.
—o— healthy plants; —•— infected plants.

Experiment II: Effect of *Ustilago bullata* on the germination and emergence of *Bromus catharticus*

Proportion and Time of Emergence of Seedlings

The final percentage of emergence of non-inoculated seeds and inoculated seeds was similar (54% and 53% respectively; $P > 0.05$; Fig. 6A). However, there was a significant difference ($P < 0.04$) in the timing and rate of emergence of the two classes of seedlings (Fig. 6B). Seedlings derived from inoculated seeds started to emerge on the sixth day after sowing, with the highest proportion of emergence

occurred on the seventh day, when 28.7% of the plants emerged. On the other hand, the first healthy seedlings appeared on the fifth day after sowing with the highest percentage of emergence occurred on the sixth (38%) and seventh (11%) days (Fig. 6B). These relatively small temporal differences had a noticeable effect on the size of infected seedlings (Fig. 7).

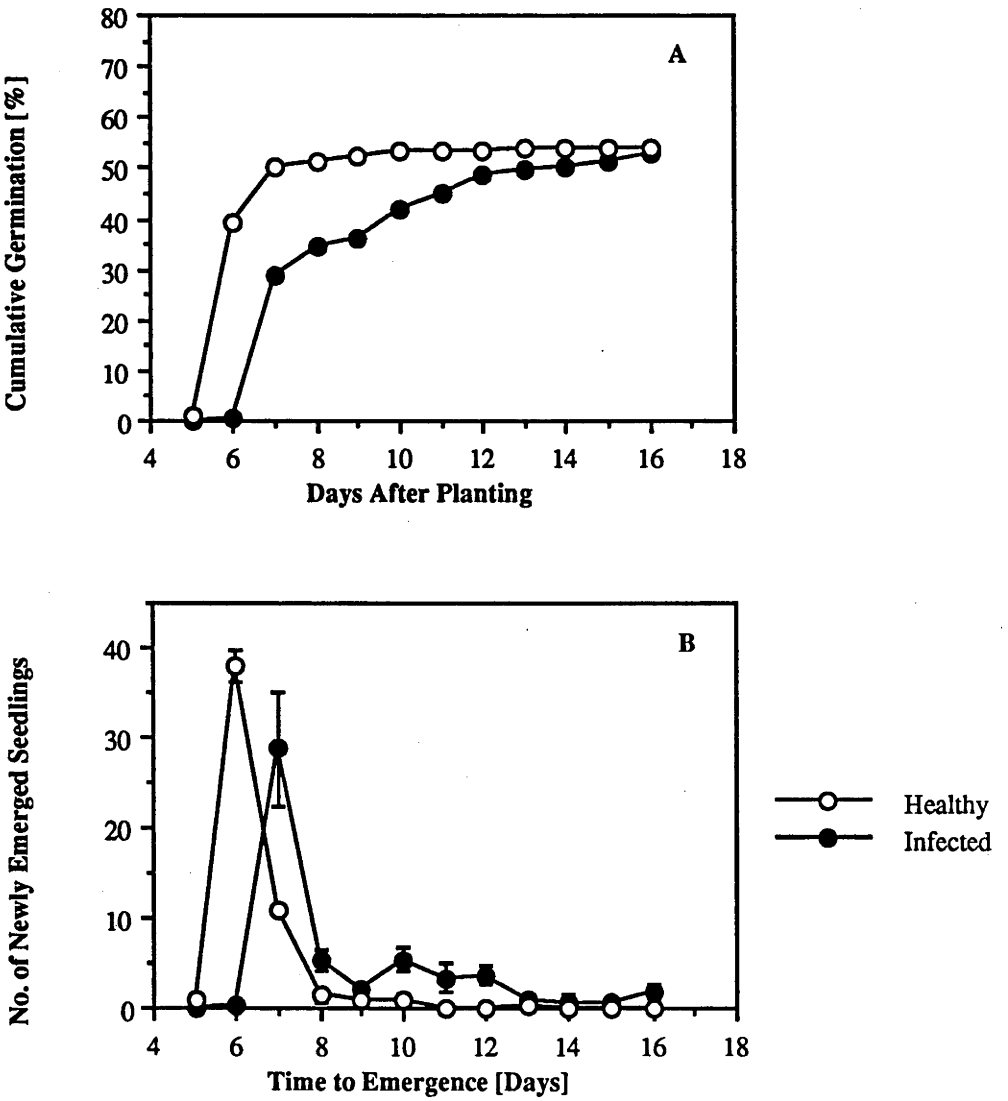
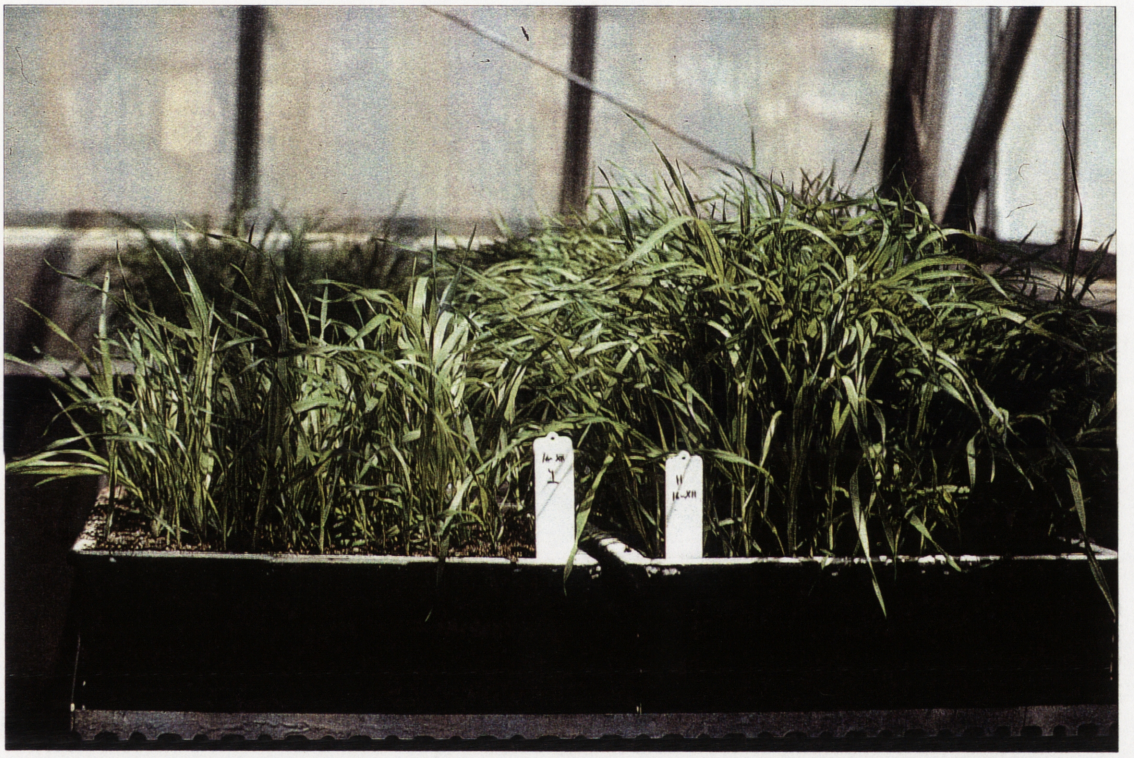


Fig. 6. Germination pattern of seed of *B. catharticus* inoculated and uninoculated with *U. bullata.* (A) Percentage of cumulative germination during 14 days, and (B) mean number of newly emerged healthy and infected *B. catharticus* seedlings per day. Vertical bars represent ± 1 SE.

Fig. 7. Comparison between healthy and infected *B. catharticus* seedlings. Infected on the left, healthy on the right.



Experiment III: Effect of smut disease: established seedlings to adults

Experiment I showed that infection by *U. bullata* affected plant size, survival (under low nutrient levels) and allocation of resources between roots and shoots. However, with the exception of the low nutrient treatment, the competitive ability of infected plants relative to healthy ones was unaffected. In addition, experiment II showed that infection can affect the timing of seedling emergence. Potentially this differential germination and establishment of individual plants could result in the development of competition / size hierarchies in which individuals that become established first (often healthy individuals) are favoured (Harper 1977). In these circumstances infected *B. catharticus* could be at a competitive / size disadvantage relative to healthy plants. With experiment III I aimed to separate out this possibility and look at the performance of equally sized individual seedlings in pure stands and in a 25% infected : 75% healthy mixture at three densities.

As with experiment I, the total dry weight of *B. catharticus* plants was significantly affected by density, frequency of infected plants and the disease status of the individuals ($P < 0.0001$). The interaction frequency x density was not significant ($P > 0.05$). Plants were smaller at high densities in all treatments. Healthy plants growing in pure stands and in mixture with infected individuals had higher dry weights than infected plants. This difference was more noticeable when plants grew at low densities (Fig. 8).

Effect of the level of nutrients

Healthy and infected plants grown at low nutrient levels were again smaller (cf. Experiment I) than equivalent plants grown at the higher nutrient level ($P < 0.001$; Table 1). With the exception of the infected monoculture at both nutrient levels, infected plants were smaller than healthy ones and the lower nutrient level was again typified by larger R/S ratios. Root / shoot ratios were substantially less than those recorded in Experiment I.

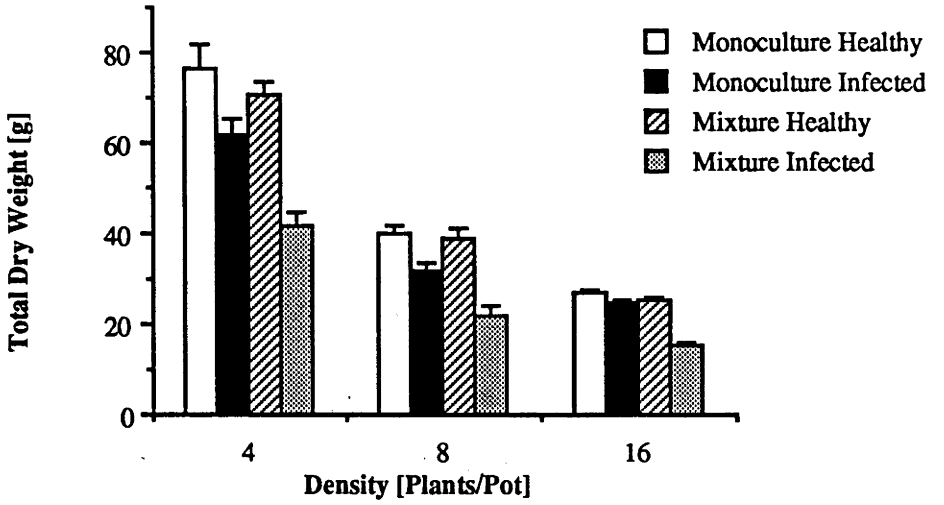


Fig. 8. Mean total dry weight of healthy and infected *B. catharticus* plants growing at three densities in monoculture and in 25% infected : 75% healthy mixtures at three densities. Vertical bars represent ± 1 SE.

The allocation of resources between roots and shoots was again (cf. Experiment I) affected by disease ($P < 0.01$) and density ($P < 0.0001$) but not by the frequency of infected plants in the pot ($P > 0.05$) or the interaction of frequency with density ($P > 0.05$; Fig. 9). At low density, infection depressed R/S ratios (more shoot), particularly when infected plants were grown in mixture with healthy plants. In contrast, at higher densities and in monoculture the R/S ratio of *B. catharticus* plants was little affected by *U. bullata* (Fig. 9). Root/shoot ratios recorded in this trial were substantially lower for healthy plants than in Experiment I. Those of infected plants were noticeably lower only when plants were grown at 4 plants per pot.

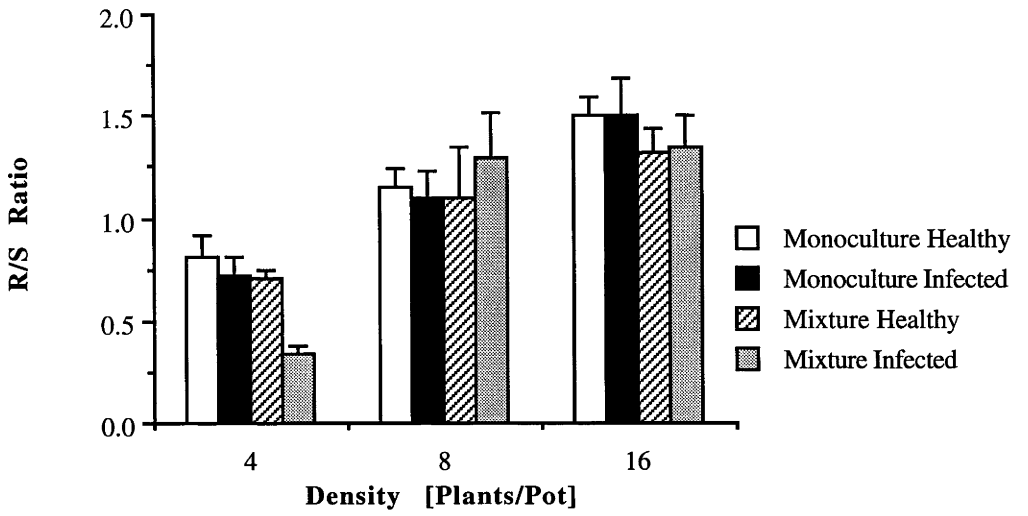


Fig. 9. Root / shoot ratios for healthy and infected *B. catharticus* plants in monoculture and in a 25% infected : 75% healthy mixture at three densities. Vertical bars represent $\pm$ 1 SE.

In contrast, the effects infection by *U. bullata* had on the growth and competitive ability of infected plants were unexpected. Infection reduced the growth rate and overall size of infected individuals regardless of planting density or the relative frequency of healthy and infected individuals in mixtures (Fig. 3). Most surprisingly though, these reductions had virtually no effect on the relative competitive ability of healthy and infected *B. catharticus* plants with both healthy and infected individuals competing equally for the same limiting resources ($k_{hd} = 1 = 1/k_{dh}$) even when the intensity and timing of the outset of competition was changed dramatically through a 4 -fold change in plant density. Indeed it was only when competitive pressures were further manipulated by reducing soil nutrient levels that healthy plants showed even a modest increased competitive ability relative to infected ones ($K_{hd} = 1.52$, $K_{dh} = 0.66$; Fig. 4). In other plant-pathogen associations it has also been observed that environmental

stress may result in changes in the outcome of intraspecific competition. Thus, under drought conditions the competitive ability of *Senecio vulgaris* infected by the non-systemic rust *Puccinia lagenophorae* is reduced, but not as much as that occurring under well-watered conditions (Paul and Ayres 1987b). In addition, this disease significantly reduces plant growth under relatively nutrient-rich conditions, but not under nutrient poor conditions (Paul and Ayres 1986b).

A wide range of other pathogens including those typically classified as ***r*** type strategists also cause substantial reduction in the size of infected individuals (eg. *Erysiphe graminis hordei* infecting barley; Brooks 1972). However, the distinctly different feature of the current interaction is the subtlety of its effect. Unlike more typically ***r*** type interactions the effect of *U. bullata* on the size of *B. catharticus* individuals is not translated into an immediate large effect on competitiveness.

Published patterns of competition in mixtures of healthy and infected plants show a diversity of patterns. For ***r*** strategists, particularly those which affect vigour, but do not kill their hosts immediately, such as obligate foliar pathogens, infection may result in markedly reduced competitive ability for the diseased hosts. The non-systemic rust *Puccinia chondrillina*, for example reduces the competitive ability of disease susceptible genotypes (k_{sr}) of *Chondrilla juncea* ($k_{sr} = 0.73$, $k_{rs} = 1.37$), which in absence of disease have similar competitive ability to that of the resistant (k_{rs}) genotypes ($k_{sr} = 1/k_{rs} = 1$; Burdon *et al.* 1981). *Puccinia lagenophorae* infecting *Senecio vulgaris* has an even more pronounced effect in reducing the competitive ability of infected (d) plants (k_{dh}) in mixture with uninfected (h) individuals ($k_{hd} = 3.32$, $k_{dh} = 0.39$; Paul and Ayres 1986a), however in this case healthy and infected plants apparently were not competing for exactly the same resources ($k_{hd} \neq 1/k_{dh}$).

In contrast, the effects of infection by systemic pathogens or endophytes on the competitive ability of their hosts appear to form a continuum. This ranges from reductions in the competitive ability of infected plants such as the systemic rust *Puccinia minussensis* infecting *Lactuca sibirica* (Wennström and Ericson 1992; Wennström 1994) or the endophyte *Acremonium lolii* infecting *Lolium perenne* (Marks *et al.* 1991); toward those fungi having no effect [eg. *Silene dioica* infected by the systemic smut *Microbotryum violaceum* (Carlsson and Elmqvist 1992) and *Actaea spicata* infected by the systemic smut *Urocystis carcinodes*

(Wennström 1994)], to those enhancing the competitive abilities of infected individuals, for example the associations involving *Acremonium coenophialum* infecting *Festuca arundinacea* (Marks *et al.* 1991), *Atkinsonella hypoxylon* infecting *Danthonia spicata* (Clay 1984) or the systemic rust *Puccinia pratensis* infecting *Pulsatilla pratensis* (Wennström and Ericson 1991; Wennström 1994).

However, in most of these studies, competitive patterns are inferred from observations of patterns in changes in dry matter production or flower production. Moreover, healthy and infected plants are often only grown in a single proportion mixture. However as my results show such data alone fails to directly assess the relative competitive relationships, while relative crowding coefficients gave indication of the equal competitive abilities of healthy and infected plants. In the current study plants were grown at different densities, in monocultures and in mixtures of varying proportions of healthy and infected plants, as well as under different nutrient supply, showing that such traits are important in the outcome of competition between healthy and infected *B. catharticus* plants.

Accompanying these effects on plant size and competitive ability were major changes in the allocation of resources within infected plants such that the proportion of resources allocated to roots was very substantially less in infected individuals than in healthy ones (Fig. 5 and 9). Reduction in growth and root to shoot ratios are not uncommon for many obligate foliar diseases, for example *Puccinia lagenophorae* infecting *Senecio vulgaris* (Paul *et al.* 1990) and *Puccinia chondrillina* infecting *Chondrilla juncea* (Burdon *et al.* 1981). Such effects could have significant consequences for the survival of infected individuals in drought conditions (fewer roots in infected individuals) or other harsh abiotic conditions, or may, as it was detected in experiment I, affect their competitive ability under low nutrient conditions. The cause of the shifts in root/shoot ratio are unclear, though if the fungi are principally active in shoot tissues they may alter the source-sink- metabolite relationship of the plant, and if they are active in apical meristems they may disrupt the normal control of development. Root/shoot ratios generally decline as plants increase in size, so a reduction in growth rate could keep plants in a more "juvenile" condition.

I found that mortality among healthy and infected adult plants was very similar. However, previous agronomic studies have shown that survival of smut-infected *B. catharticus* is significantly reduced after flowering (Falloon 1976),

presumably because of additional stress in the field. The infected plants in my experiments were much smaller and this may have given them much reduced survival in the long-term if they were subject to adverse conditions. *U. bullata,* requires live plants for survival, so that any increase in aggressiveness that results in the death of the plant, immediately results in the death of the pathogen. This increased risk of mortality following pathogen reproduction may reflect the outcome of selective pressures favouring pathogen behaviours that minimize the damage inflicted on hosts prior to flowering. Clearly death of infected hosts prior to flowering imposes a very strong selection pressure favouring such a deferral of damage.

On the other hand, during the early phases of the host-life cycle, infection by seed-infecting systemic-smuts, has variable effects on the germination and seedling establishment of their hosts. These effects range from those smuts having little effect, for example a study with *U. bullata* infecting *Bromus willdenowii* showed that establishment of infected (43%) and healthy seedlings (45%) in stands was very similar (Luttrell and Craigmiles 1961); to others that have a much more pronounced effect such as the Karnal bunt of wheat, *Neovossia indica,* where severe seed infections can reduce germination by 67% (Singh and Krishna 1982). In contrast, infection of some grasses by endophytic fungi results in increased germination and vigour. Thus *Lolium perenne* and *Festuca arundinacea* individuals infected by the fungal endophytes *Acremonium loliae* and *A. coenophialum* grow faster than uninfected individuals (Clay 1987).

Infection by *U. bullata* affected the rate of seedling emergence (experiment II). In particular, seedlings from inoculated seed tended to emerge one day later than from uninoculated seed. This suggests that infection process delays early seedling growth, though an inhibitory effect on germination is also possible. However, the final percentage of seedling emergence was unaffected by smut disease (Fig. 6), which reinforces the idea that evolution may favour attributes that minimize the damage to *B. catharticus* by *U. bullata,* even at the early stages of the life cycle, so that the fitness of this smut pathogen is raised.

Smuts can reduce the vigour of infected seedlings growing under particular environmental conditions. In particular, host density is likely to have a strong effect on the vigour of the infected seedlings. For example Doling (1964) found that barley seedlings infected by *U. nuda* were very much less vigorous than non-infected seedlings, particularly when grown at high densities. However, my

results suggest that (at least for the densities used here) this is not the case for the association between *U. bullata* and *B. catharticus*, since the low mortality and equal competitive ability of healthy and infected plants detected in experiment I implies that vigour of infected seedlings was unaffected even though initial size might be smaller. It is possible that part of the difference between *U. bullata* and *U. nuda* results from their mode of infection - the latter infecting the developing ovule rather than invading at germination. The similarity of results between experiments I and III suggests that disease does not have an important effect during the very early phases of seedling establishment, so that survival and competitive abilities of the adult plants were unaffected, although plant size was strongly reduced by infection (≈ 1/3 size).

However, these differences in size are potential very important as they reflect a reduced storage capacity of the infected plants and therefore a likely increased vulnerability to stressful field conditions. My experiments were carried out in a glasshouse where severe stress which might damage or kill them was minimized. In the field, size may confer advantages in relation to stress, especially the size of organs from which recovery after grazing might occur.

Nevertheless, although experiments I and III showed similar trends, there were differences in the magnitude of these effects. For example root to shoot ratios were smaller in experiment III (Fig. 9) than in experiment I (Fig. 5). Some of these differences may be the result of the more equal-sized plants used in experiment III while in Experiment I differential establishment of individual plants could have resulted in the development of size hierarchies and therefore more variability in some physiological responses.

The association between *U. bullata* and *B. catharticus* appears to combine selective elements from both ends of the ***r*** - ***K*** continuum. The low effect of the pathogen on the emergence of seedlings as well as the lack of effect on the survival of the adults and competition are characteristics that suggest a close relationship, where selective forces have apparently favoured low aggressivity for *U. bullata*, placing this association closer to the ***K*** end. In contrast, the greatly reduced host size, the high production of spores and the complete destruction of the host inflorescences by the pathogen which could result in a decreased number of possible hosts in the next generation, and the change in resource allocation which may well make hosts more vulnerable to adverse

conditions, all indicate that this interaction is still far from benign from the plant's point of view.

CHAPTER 3

Effects of the flower smut *Ustilago cynodontis* (Pass.) Henn. on growth, survival and competitiveness of *Cynodon dactylon*. (L.) Pers.

INTRODUCTION

Systemic fungi, ranging from smuts to endophytes, have a variety of effects on their hosts. In many associations involving dicotyledonous or monocotyledonous host plants, infection results in partial or complete sterility (Alexander and Maltby 1990; Carlsson and Elmqvist 1992; Jennersten *et al.* 1983; Clay 1991; Wennström and Ericson, 1991). For endophytic associations there is good evidence that this host castration involves a fitness trade-off with infected hosts showing increased growth and/or survival (eg. *Balansia cyperi* infecting *Cyperus virens*; Clay *et al.* 1985) or competitive ability (eg. *Festuca arundinacea* infected by *Acremonium coenophialum*; Marks *et al.* 1991). These effects are not exclusively restricted to mutualistic endophytic associations. Similar effects have been reported in a few studies involving more pathogenic systemic fungi (eg. systemic rusts or smuts). Thus, there is some evidence of increased vigour in individuals of the perennial herb *Pulsatilla pratensis* infected by the rust *Puccinia pulsatillae* (Wennström and Ericson 1991) and the competitive ability of the perennial plant *Silene dioica* seems to be unaffected by the systemic smut *Microbotryum violaceum* (Carlsson and Elmqvist 1992). [Although the closely related interaction between *S. alba* and *U. violacea* does affect survival under harsh conditions; Thrall and Jarosz 1994]. This suggests that similar effects to those of endophyte interactions can occur in other associations.

The highly variable response of host species to systemic diseases seems to be related not only to the identity of the pathogen itself, but also to growth patterns exhibited by the hosts. Thus systemic diseases of dicotyledonous herbs with strong lateral growth (stolons, rhizomes, etc.), have been shown to have a marked negative effect on survival and competitive ability (eg. the smut fungus *Urocystis trientalis* infecting *Trientalis europaea,* and the rust *Puccinia*

minussensis infecting *Lactuca sibirica;* Wennström 1994), while similar diseases of clonal plants with weak lateral growth seem to have a more benign effect even to the extent of increasing survival and competitive ability (eg. *Pulsatilla pratensis* infected by the rust *Puccinia pratensis*; Wennström 1994).

In the previous chapter I showed that while infection by the smut *Ustilago bullata* on the perennial tufted grass *Bromus catharticus* depresses the rate of emergence of seedlings and size of plants it appeared to have very little to no effect on the survival and competitive ability of plants. In this chapter I present data on the interaction between the rhizomatous grass *Cynodon dactylon.* (L.) Pers. and the systemic smut fungus *Ustilago cynodontis* (Pass.) Henn. In particular I wanted to test the hypothesis that a systemic smut disease may more severely affect establishment and especially, growth, survival and competitiveness of a clonal rhizomatous perennial species of grass. Since this may be influenced by resources for plant growth, I also examined how the level of nutrients in the soil could affect this interaction.

The *Cynodon dactylon -Ustilago cynodontis* host-pathogen system

Cynodon dactylon (L.) Pers. is a clonal, perennial grass with slender, more or less prostrate stems that spreads by lateral growth of rhizomes and branching stolons. The species is an almost cosmopolitan weed of warmer regions of the world, and may have been present in Australia before European settlement. In Australia *C. dactylon* grows on a wide range of soil types and has been used widely as a rough lawn (Lamp *et al.* 1990; Burbidge 1966).

This grass is affected by the systemic floral-smut fungus *Ustilago cynodontis* (Pass.) Henn. All inflorescences produced by infected plants are smutted. In laboratory trials infection was observed to occur at the seedling stage from spores contaminating the seed coat or present in the soil. The detailed life cycle for this smut species is unknown, but is believed to follow that of other species of *Ustilago* which infect their hosts at the seedling stage - via infection hyphae formed after fusion of sporidia from germinated spores (eg. *U. bullata;* Falloon *et al.* 1988). As the host plant grows, the smut-fungus spreads throughout the tissues of the plant, remaining asymptomatic throughout the vegetative phase of the life cycle. Typical smut symptoms appear at flowering when glumes and ovaries are replaced by a black, powdery mass of teliospores (Fig. 1).

Fig. 1. The effects of infection by the floral-smut fungus *Ustilago cynodontis* on *Cynodon dactylon*. Left: smutted heads; right: healthy inflorescences.

METHODS

Effect of *U. cynodontis* on germination of *C. dactylon*

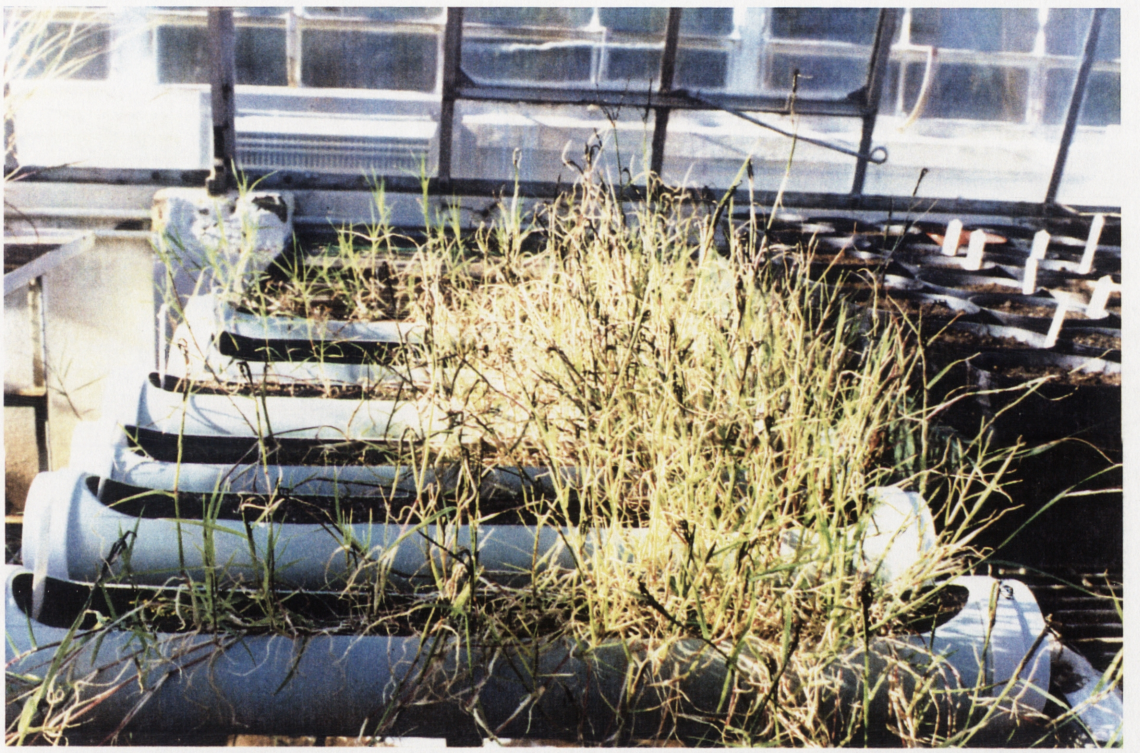
The effect of infection by *U. cynodontis* on germination of *Cynodon dactylon* seedlings was examined in an experiment involving six levels of inoculation using a mixture of teliospores from several host plants. 0.5 g lots of seed were vacuum-inoculated (for 15 minutes) in 0.008, 0.004, 0.002, 0.001, and 0.1 g of *U. cynodontis* teliospores / litre of distilled water. An uninoculated control was provided by using sterilised distilled water instead of the fungal spore suspensions. Following this treatment the seed was left in the suspension for a further 24 hrs. One hundred inoculated and control seed were separately sown in plastic flats filled with standard potting mix. Each treatment was replicated three times. The flats were kept in a naturally lit glasshouse, (18 - 24°C) and the time to emergence of each seedling recorded over 20 days. Seedlings were considered to have germinated when the plumule was visible above the soil surface.

Data analysis: The effect of smut disease on germination and establishment was analysed using logistic regression (McCullagh and Nelder 1989). The response variables were the total number of seedlings at the end of the experiment and number of new seedlings per day as proportions of the total number of seed. Predictive variables were disease status (smut inoculated or uninoculated), inoculum levels and time (days).

Effect of smut disease and nutrient levels on the growth of stolons

For this experiment 0.5 g of *C. dactylon* seed were vacuum-inoculated and then left to stand in a 0.004 g/litre spore suspension for 24 hours. Following these treatments the seed was sown in flats containing either high (standard potting mix) or low nutrient soil respectively. Low nutrient soil was made by mixing standard potting mix with sand in a ratio of 1:3. One week after emergence, 20 inoculated and 20 healthy seedlings were individually transplanted to the end of a plastic tray (50 cm long, 10 cm wide and 10 cm deep; Fig. 2) filled with either high nutrient or low nutrient soil. Thus, ten replicates were established for each treatment. The lateral growth of the primary stolons produced by these seedlings was measured daily for a period of 15 days and then weekly for a further 35

Fig. 2. Photograph showing pots used to assess *C. dactylon* stolon growth. Plants have grown from right to left.



days. These data were then used to obtain relative growth rates using the following formulae:

$$GR = (\log FL - \log IL) / D$$

Where GR is the growth rate, FL is the length of stolons at the end of the experiment, IL is the initial length of the stolon and D is the total number of days.

Data analysis: The effect of infection by *U. cynodontis* and the level of nutrients in the soil was analysed by two-factor analysis of variance (ANOVA; Zar 1984) on the growth rate of stolons.

Disease transmission along stolons

After growth rates were obtained in the previous experiment, the infected plants were kept to determine the extent of disease transmission along the stolon. From each plant the longest stolon was selected and cut into 10 equal sections. For each section, the position from the parent plant was recorded. Each section was then planted in low or high nutrient soil depending of the kind of soil from which it originated. These sections formed roots and were allowed to grow until flowering occurred in order to determine their disease status.

Data analysis: Disease transmission along the stolons and the effect of the level of nutrients in the soil on disease transmission was analysed using logistic regression (McCullagh and Nelder 1989). The response variable considered was the disease status of the stolon segments and differences in this variable were explained in terms of distance from the parent plant and nutrient levels (high or low).

Effect of *U. cynodontis* and seedling density on the competitive ability of *C. dactylon*

In order to determine the effect of infection by *U. cynodontis* on the performance of *C. dactylon*, I carried out two experiments. The first experiment (I) explored the effect of smut disease on the overall growth of *C. dactylon* and its variation in a gradient of densities and in a mixed stand of infected and healthy plants, and also the effect of soil nutrient level. The second experiment (II) focused on

the effects of smut disease on the competitive ability of *C. dactylon* by determining relative crowding coefficients for healthy and infected individuals. Considering the results from experiment I, I decided to carried out this more detailed assessment of the competitive interaction between healthy and infected *C. dactylon* at a density of 8 plants per pot. At that density it was clear that competition was occurring since the size of individual healthy and infected plants was markedly smaller at that density than at 4 plants/pot.

For both experiments (I and II), *C. dactylon* seeds were either inoculated with a 0.004g/l spore suspension or placed in distilled water (healthy control), planted in separate flats, and kept in a naturally lit glasshouse until germination and establishment occurred. Once established, inoculated and control seedlings that were visually of equal size were planted out according to the experiment.

Experiment I. Seedlings were planted at three different densities (4, 8 and 16 plants per pot) in healthy or infected monocultures and in a 25% infected : 75% healthy mixture in 15 cm diameter pots filled with potting mix. A complementary treatment examining the effect of low and high nutrient soil was carried out at the same time. For this, a further set of infected and healthy seedlings were planted at a density of 16 plants per pot at three percentages of infection (0, 25 and 100% of infected plants per pot) in soil with low nutrients (75% sand and 25% potting mix). All pots were kept in a naturally lit glasshouse at 18-24 °C. Plants were watered daily to field capacity. After 4 months the experiment was harvested (by this time all individuals had flowered thus revealing their disease status); the roots washed clean of soil and all plants separately dried at 60°C for one week. Shoot and root weights were recorded separately and, in addition, the number of dead individuals per pot was scored.

Experiment II. Seedlings were planted at a density of 8 plants per pot and 5 frequencies of infection (0, 25, 50, 75 and 100% infected plants per pot). A complementary treatment examining the effect of low nutrient soil (75% sand : 25% potting mix) on the performance of this species was carried out at the same time. All pots were kept in a naturally lit glasshouse at 18-24°C. Plants were watered daily to field capacity. After 4 months the experiment was harvested in the same manner as for experiment I.

Data analysis: In order to determine the effect of smut-disease, density of plants, nutrient level and the proportion of infected plants per pot on the growth

of *C. dactylon* plants, an unbalanced linear mixed model was used and so a restricted maximum likelihood estimation (REML) was applied (Engel 1990). Total, shoot and root plant dry weight as well as root/shoot [R/S] ratios were log transformed prior to the analysis. Survival was analysed using logistic regression (McCullagh and Nelder 1989).

For experiment II, the total yield of healthy and infected plants growing in monoculture and mixtures was obtained and a description of the effects of smut-disease on *C. dactylon* performance was achieved by using maximum-likelihood estimates for the relative crowding coefficients (K_{hd} and K_{dh}) of the non-linear de Wit competition model (Machin and Sanderson 1977). As described in the previous chapter, relative crowding coefficients describe the competitive relationships between two co-occurring species, genotypes or treatments such that values of k greater than unity imply that the species or treatment concerned is a better competitor than the species or treatment with k values less than unity. As before (Chapter 2) the data are sequentially fitted to three submodels [$k_{hd} \neq k_{dh}$; $k_{hd} = 1/k_{dh}$; $k_{hd} = 1/k_{dh} = 1$]. The most appropriate submodel is then selected on the goodness of fit of the data to the models.

RESULTS

Proportion and Time of Emergence of Seedlings

Inoculated and uninoculated *C. dactylon* seedlings began to emerge on the fifth day after sowing (Fig. 3). Most of the seedlings emerged on the fifth and sixth day after sowing and differences among treatments were not significant ($P > 0.05$). The final percentage of emergence of seedlings (84%) also did not differ significantly ($P > 0.05$; Fig. 3).

Effect of smut disease on the growth of stolons

Growth rates were nearly linear over 25 days, then accelerating in healthy plants while slowing in infected individuals. The level of nutrients in the soil did not significantly affect the growth of stolons ($P > 0.05$). However, the overall growth rate of stolons was significantly reduced by the smut pathogen ($P < 0.0001$). On average, the growth rate of infected plants was 1.64 cm per day while for healthy plants was double that at 3.25 cm per day (Fig. 4). The interaction between nutrient level x disease status was not significant ($P > 0.05$).

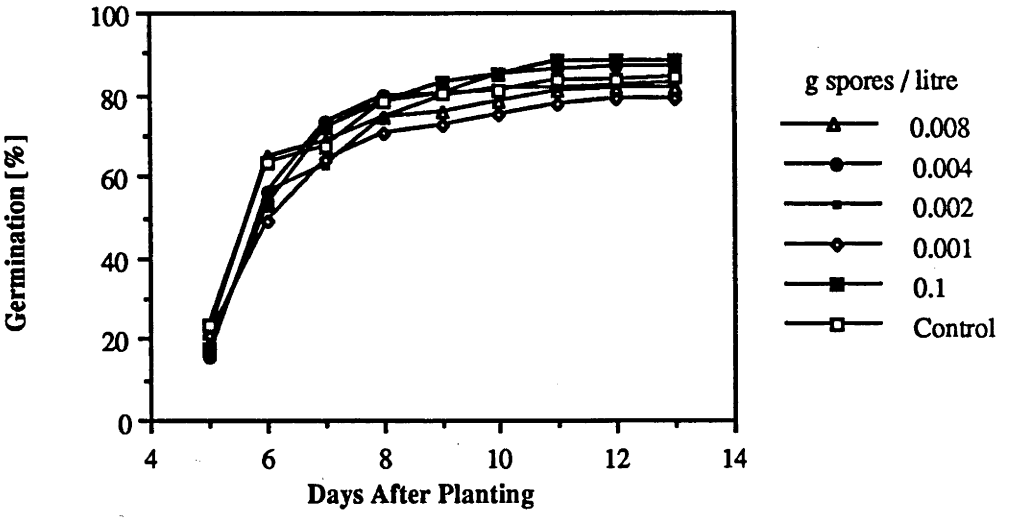


Fig 3. Cumulative percent germination of healthy and infected seedlings of *Cynodon dactylon* during 12 days, in relation to the concentration of spores applied to seeds.

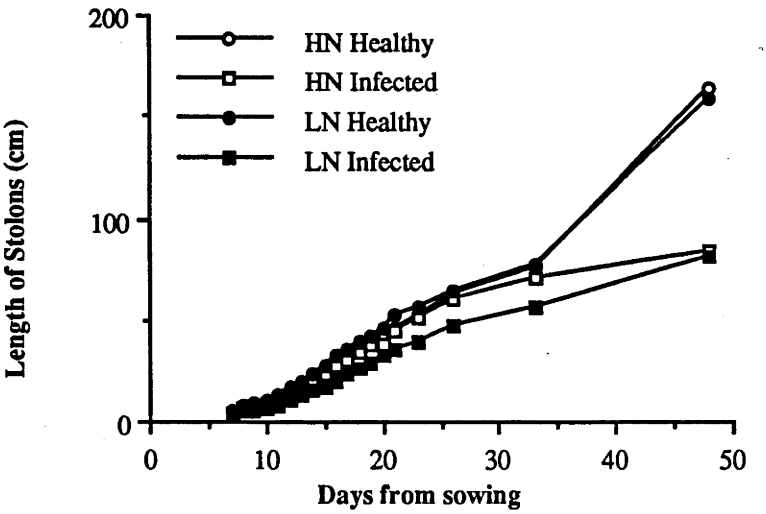


Fig. 4. Cumulative stolon growth (cm) of healthy and infected plants of *C. dactylon* at high (HN) and low (LN) nutrient levels.

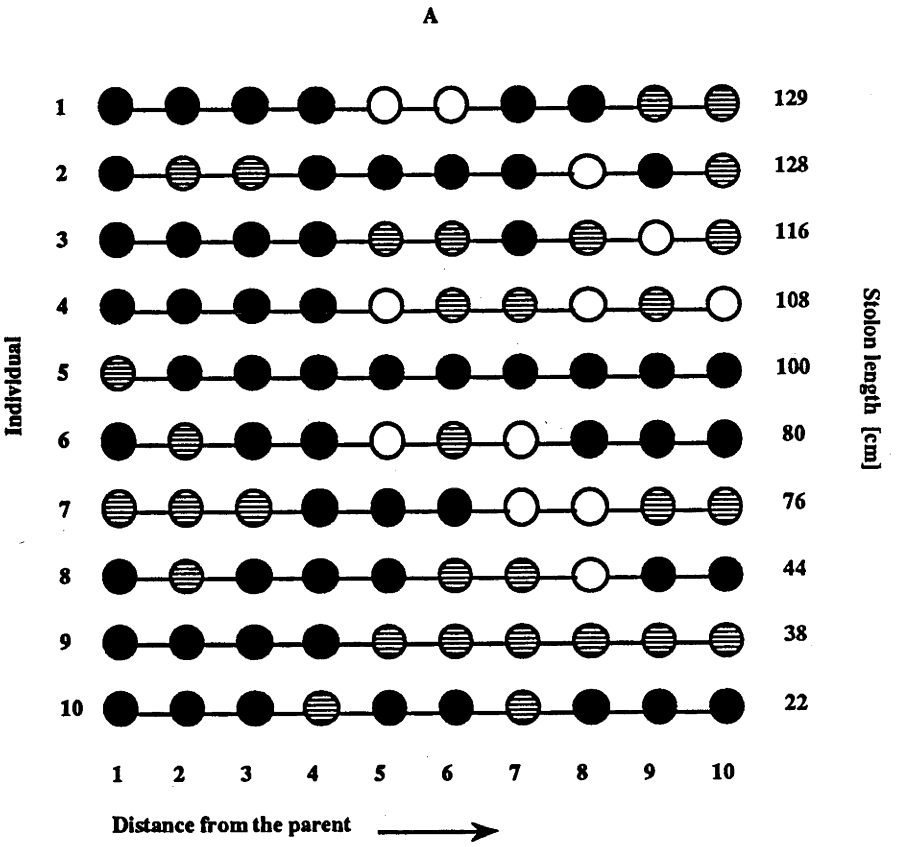
Disease transmission along stolons

At both high and low nutrient levels the transmission of the smut fungus along stolons was uneven. In both situations, the pathogen was clearly able to grow at a similar rate to that of the host itself as segments cut from the ends of the longest stolons were infected (Fig. 5A and 6A). However, in a few cases such stolons were disease - free [eg. high nutrient 3 and 4 (Fig. 5A); low nutrient 1 (Fig. 6A)] suggesting that rapid growth may provide a means of purging the disease from the plant. In addition, however, in both the high and low nutrient treatments some stolon segments were healthy even though segments on either side were diseased (eg. high nutrient, 1, 2, 8; low nutrient 1, 2, 4; Fig. 5A and 6A). Obviously, the pathogen must have been present in these segments at one time but apparently has since died.

At high nutrient levels, the number of stolon sections not becoming infected rose from none in the first 30 cm from the parent to up to three in further sections (Fig. 5B). Similarly, at low nutrient levels the number of healthy stolon sections rose from none in the first 50 cm to three at 60 cm and then to one in the furthest section (Fig. 6B). Differences between the low and high nutrient conditions were not significant ($P > 0.05$).

The survival of excised stolon segments was moderate under both nutrient levels. Segments growing at high nutrients had a mortality of 31%, and 46% at low nutrient levels. Differences in mortality among segments derived from shorter and longer stolons were not statistically significant ($P > 0.05$). In addition, at both nutrient levels, healthy segments were more common among longer than shorter stolons, however differences were not significant ($P > 0.05$). A logistic model showed significant effects ($\chi^2_1 = 10.54$; $P < 0.001$) when fitting the proportion of healthy sections and sequences of sections (1-10), however stolons had different lengths so the interpretation of this apparently does not involve growth rates.

Fig. 5. (A) Diagrammatic representation of the disease status of stolon sections produced at different distances from the parent plant and growing at high nutrient levels. ● = infected sections, ○ = healthy sections, and ⊜ = dead sections . (B) Histogram of number of individual segments infected or healthy at varying distances along the stolons.


A
Individual
1
2
3
4
5
6
7
8
9
10
129
128
116
108
100
80
76
44
38
22
Stolon length [cm]
1 2 3 4 5 6 7 8 9 10
Distance from the parent

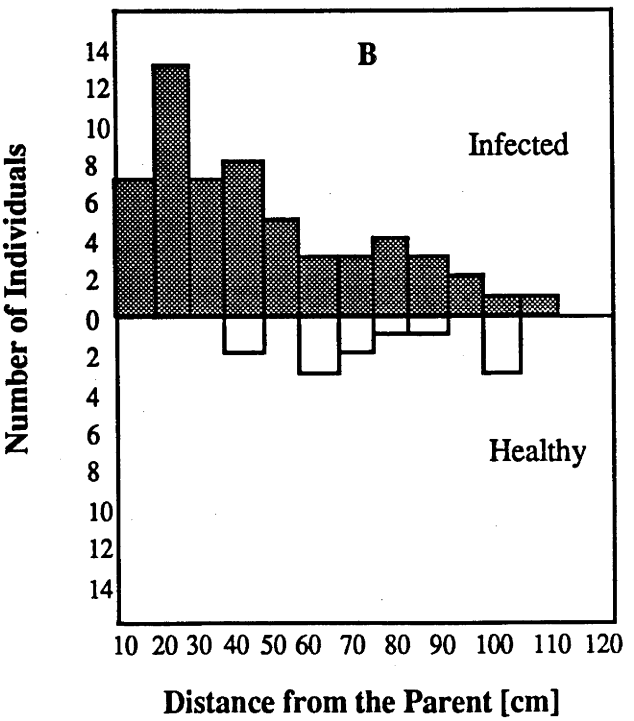
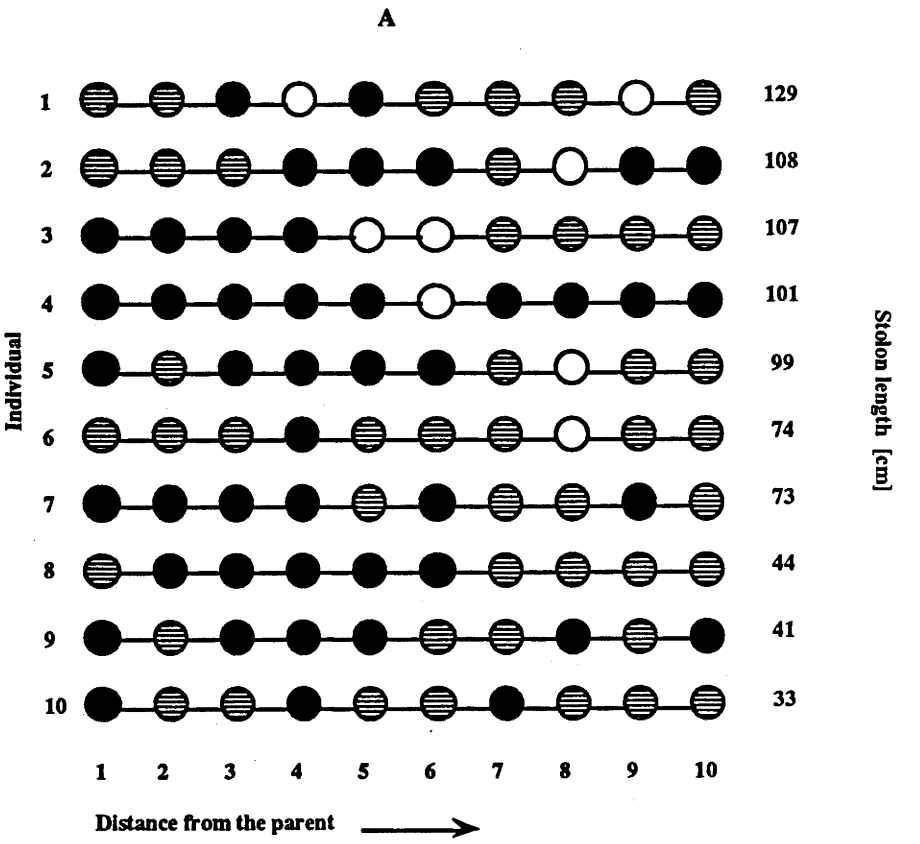
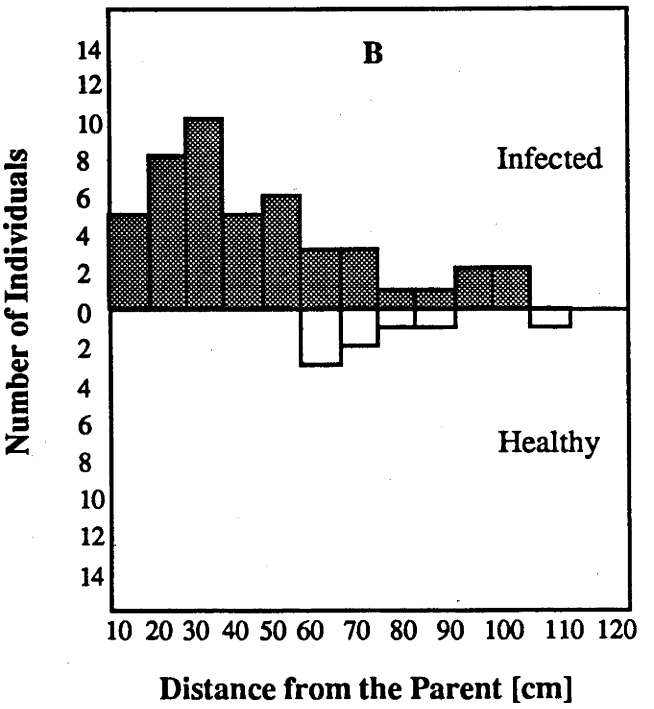

B
Infected
Healthy
Number of Individuals
14 12 10 8 6 4 2 0 2 4 6 8 10 12 14
10 20 30 40 50 60 70 80 90 100 110 120
Distance from the Parent [cm]

Fig. 6. (A) Diagrammatic representation of the disease status of stolon sections produced at different distances from the parent plant and growing at low nutrient levels. ● = infected sections, ○ = healthy sections, and ⊜ = dead sections . (B) Histogram of number of individual segments infected or healthy at varying distances along the stolons.


A
Individual
1
2
3
4
5
6
7
8
9
10
129
108
107
101
99
74
73
44
41
33
Stolon length [cm]
1 2 3 4 5 6 7 8 9 10
Distance from the parent


B
Infected
Healthy
Number of Individuals
14 12 10 8 6 4 2 0 2 4 6 8 10 12 14
10 20 30 40 50 60 70 80 90 100 110 120
Distance from the Parent [cm]

Effect of *U. cynodontis* on the competitive ability of *C. dactylon*

Experiment I (3 densities; 2 monocultures and 1 mixed stand)

Density was the most important significant factor affecting plant performance. Total individual dry weight was higher for plants growing at low densities than plants growing at high densities, regardless of whether or not they were infected ($P < 0.003$). Indeed, the total dry weight of individuals growing at a density of 4 plants per pot, was almost three times higher than that of individuals growing at a density of 16 plants per pot (Fig. 7).

Smut disease had a marginal effect, reducing the dry weight of the plants (Fig. 7; $P < 0.03$). On the other hand, neither healthy or infected plants were significantly affected by the disease status of the plants among which they grew ($P > 0.05$). The interactions density x frequency and density x disease status were not significant ($P > 0.05$).

Effect of the level of nutrients. The level of nutrients in the soil had a strong effect on the dry weight of all plants with both healthy and infected plants growing more under a high nutrient regime ($P < 0.001$; Fig. 8). Disease did not have significant effect on the dry weight of *C. dactylon* plants when grown at low nutrient levels ($P > 0.05$), however at the high level of nutrients infected plants weighed less that the healthy ones ($P < 0.01$). The frequency of infected plants in a stand as well as the interaction frequency x nutrient, did not have a significant effect on the total dry weight of healthy and infected plants ($P > 0.05$).

Root / shoot ratio

Resource allocation within *C. dactylon* was significantly affected by disease ($P < 0.001$; Fig. 9). In monoculture there was no consistent trend with little difference between R/S ratios at 4 and 16 plants/pot. Only at 8 plants/pot did healthy plants show markedly larger R/S than infected plants (Fig. 9A). In mixtures, the situation was slightly different. Infected plants had a greater R/S ratio at 4 plants/pot, but this was reversed for 8 and 16 plants/pot (Fig. 9B). Density, frequency of infected plants and the interaction of these variables were not statistically significant ($P > 0.05$).

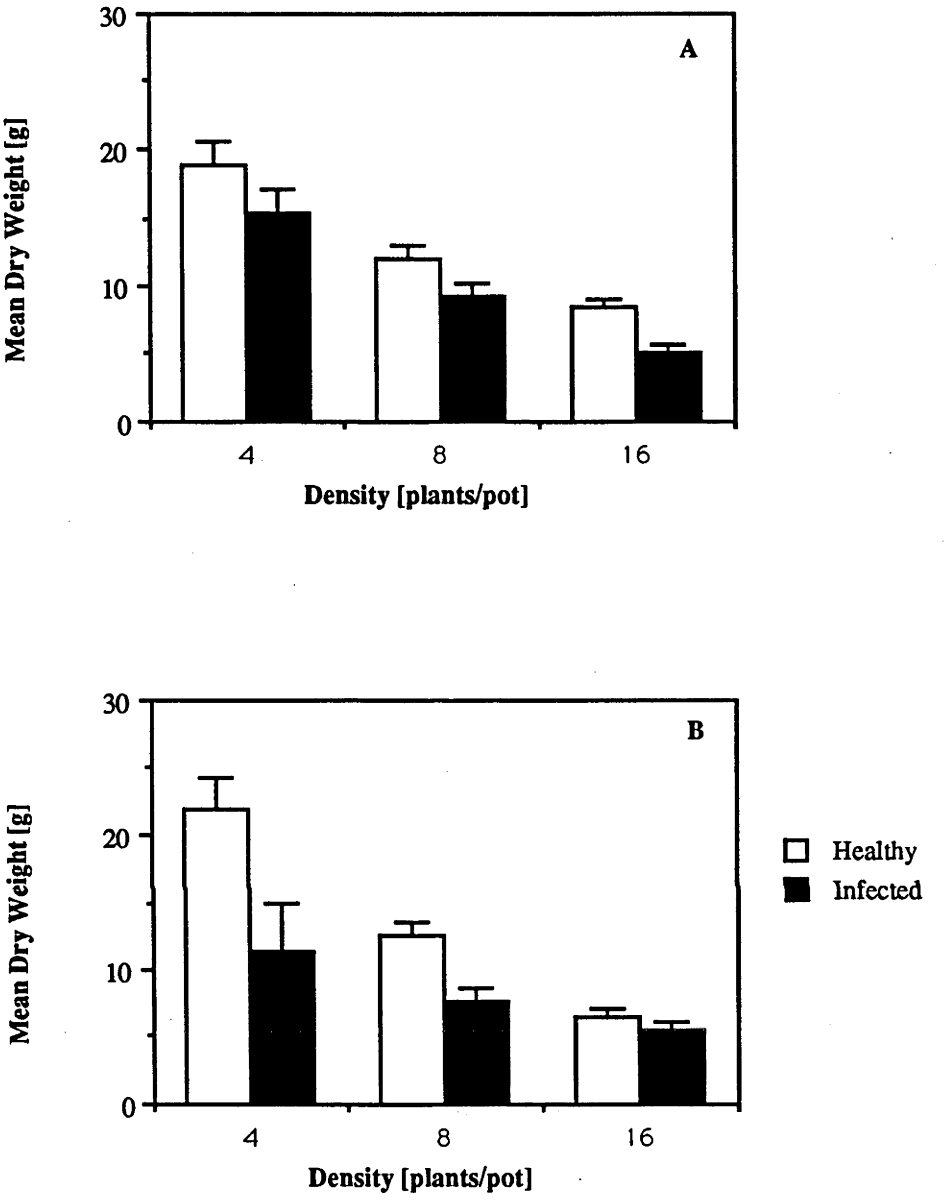


Fig. 7. Mean dry weight per plant for healthy and infected *C. dactylon* growing at three densities in (A) monoculture and (B) mixture 25% infected: 75% healthy. Vertical bars represent ± 1 SE.

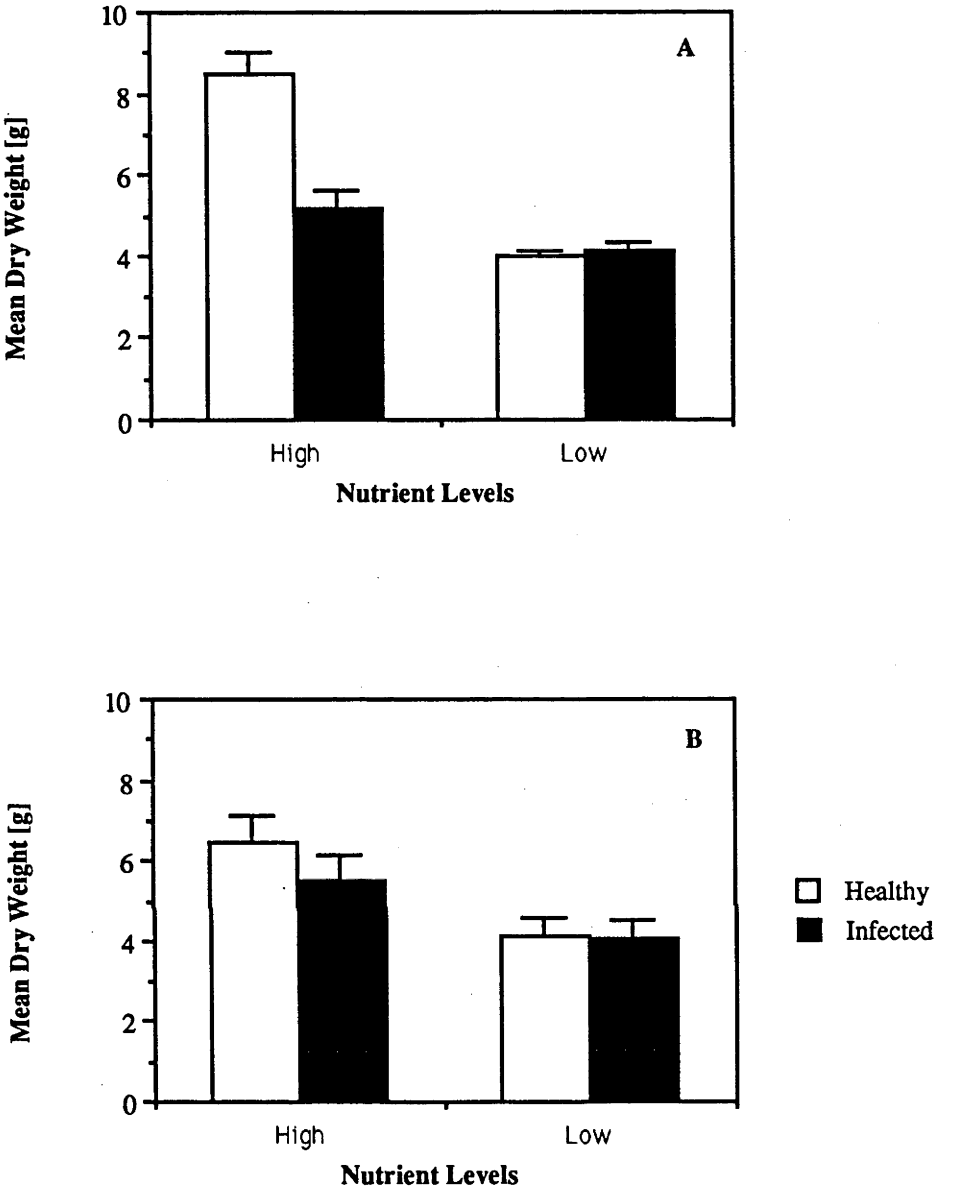


Fig. 8. Mean dry weight (g) of healthy and infected *C. dactylon* growing at two levels of nutrients in (A) monoculture and (B) mixture 25% infected: 75% healthy. Vertical bars represent ± 1 SE. [Plant density 16 plants / pot].

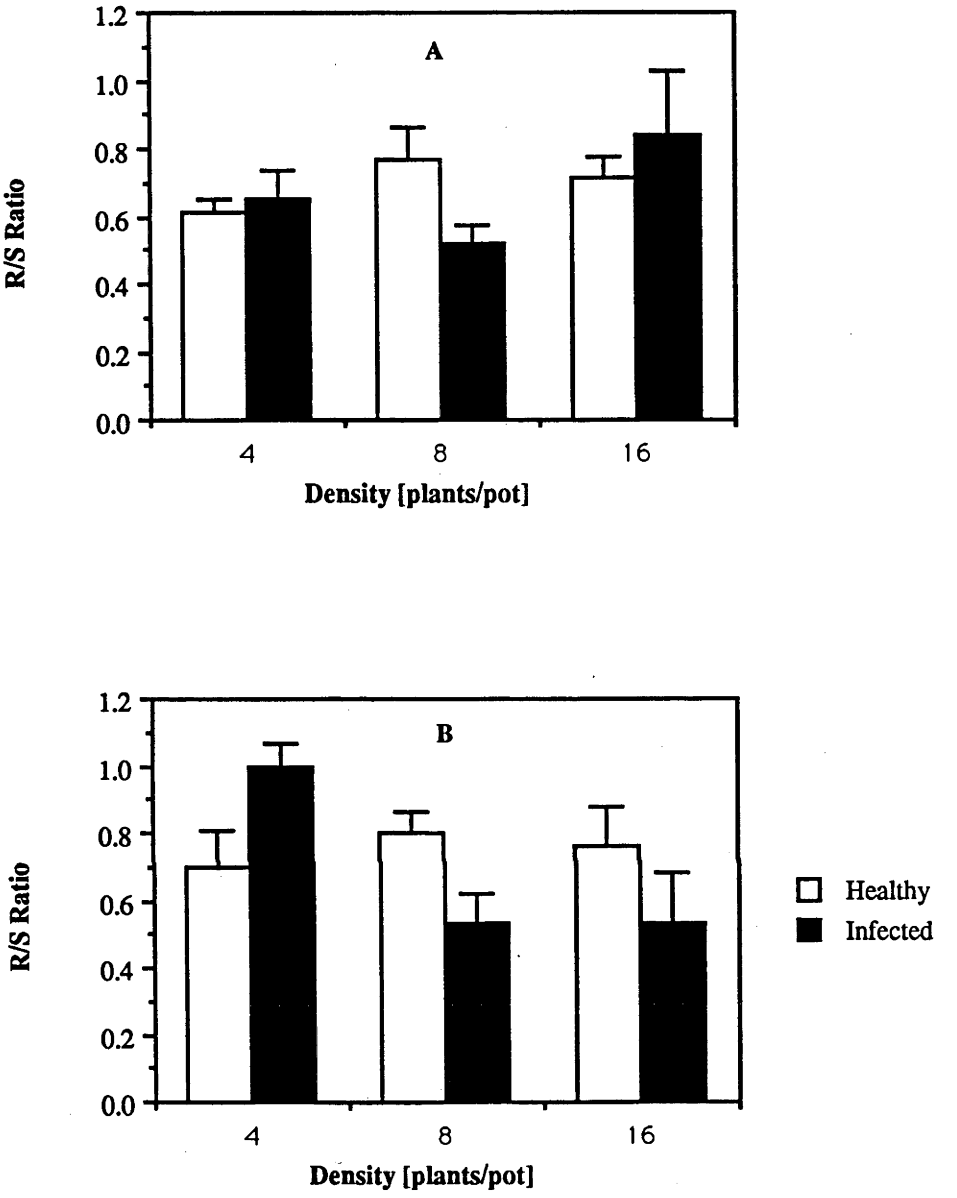


Fig. 9. Root / shoot dry weight ratio for healthy and infected *C. dactylon* plants growing at three densities, (A) in monoculture and (B) mixture 25% infected: 75% healthy. Vertical bars represent ± 1 SE.

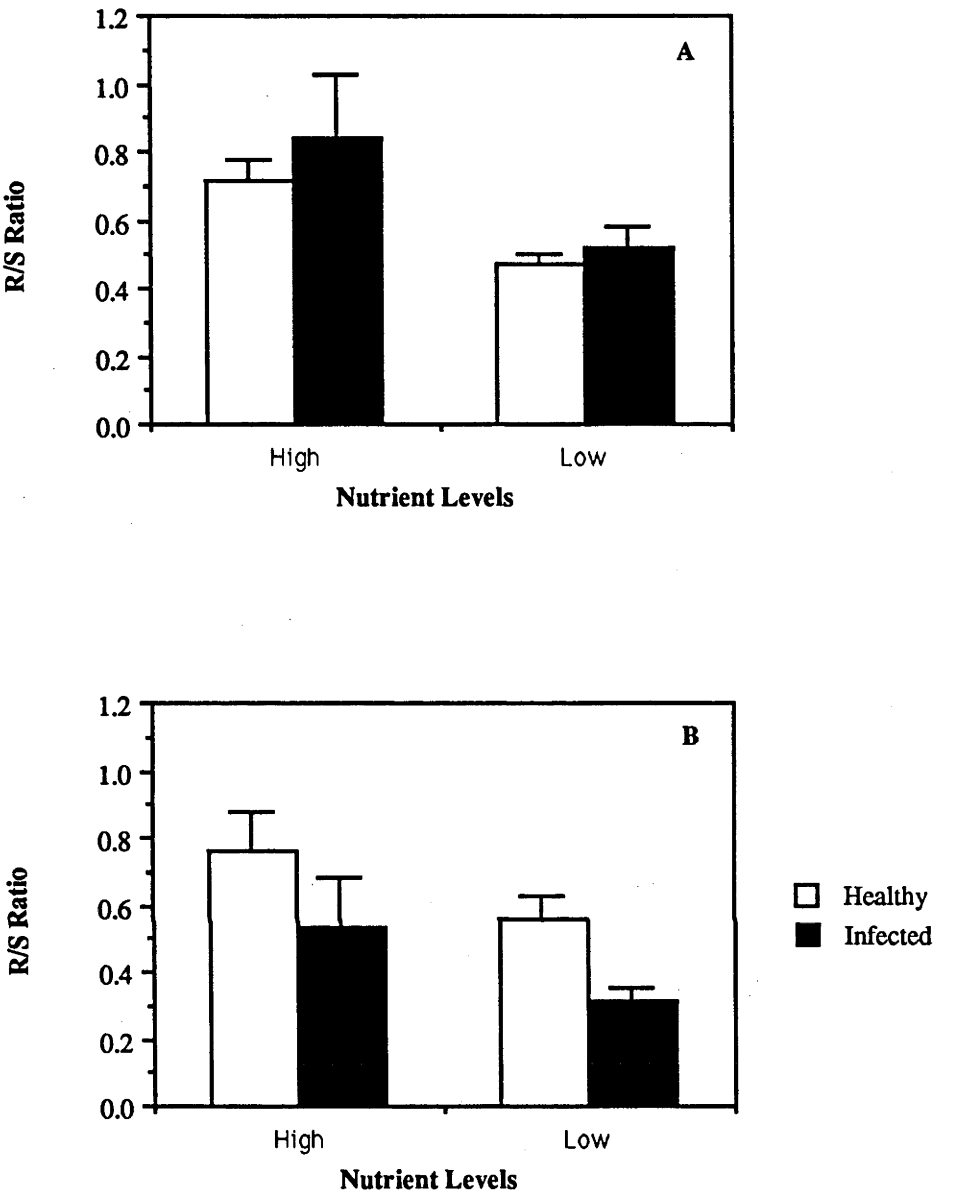


Fig. 10. Root / shoot [R/S] dry weight ratio for healthy and infected *C. dactylon* plants growing under two levels of nutrients in (A) monoculture and (B) mixture 25% infected: 75% healthy. Vertical bars represent ± 1 SE.

Effect of the level of nutrients. R/S ratios were significantly reduced by smut disease at a low level of nutrients in the soil ($P < 0.004$), but not by the frequency of infection ($P > 0.05$; Fig. 10). The interaction of these variables was not significant ($P > 0.05$). Under both nutrient levels, it was noticed that infected plants growing in mixture with healthy plants had smaller R/S ratios, than infected plants growing in monoculture while healthy plants had similar ratios.

Survival

Survival was significantly reduced by density and disease but only at the highest density (16 plants/pot; $P < 0.001$). Below this density, survival of either healthy or infected plants always exceeded 95%. When plants were crowded 16 to a pot, survival of diseased plants (77%) was significantly lower than that of healthy ones (89% ; $P < 0.001$). Survival was significantly higher for healthy and infected plants growing under high nutrient levels (84%) than for plants growing at low nutrient levels (74%; $P < 0.01$). Again at low nutrient levels, healthy plants showed greater survival than infected plants, but this difference was not statistically significant ($P > 0.05$).

Experiment II (1 density; 2 monocultures and 3 mixtures)

The effect of *U. cynodontis* on competition between healthy and infected *C. dactylon* plants in all proportion in mixtures is shown in Fig. 11. Both infection by *U. cynodontis* and low nutrient levels depressed the total dry weight of *C. dactylon* plants. Healthy plants were twice as heavy at high nutrient level as at low nutrient level. At high nutrient levels infected plants growing in monoculture weighed 30% less than the healthy individuals growing under the same conditions. While, at low nutrient levels, the dry weight of the infected plants was 40% less than that of the healthy ones (Fig. 11).

Most interestingly though, these effects of disease on plant yield were not translated into a change in competitive ability. Quantitative descriptions of the competitive performance of healthy and infected *C. dactylon* plants were determined by the relative crowding coefficient (k). These showed that at both nutrient levels, values of the relative crowding coefficients were very close to 1, so that the competition model that provided the best fit was $K_{hd} = 1/K_{dh} = 1$. This result suggests that despite the presence of the pathogen healthy and infected plants competed with equal efficiency for resources (Fig. 11).

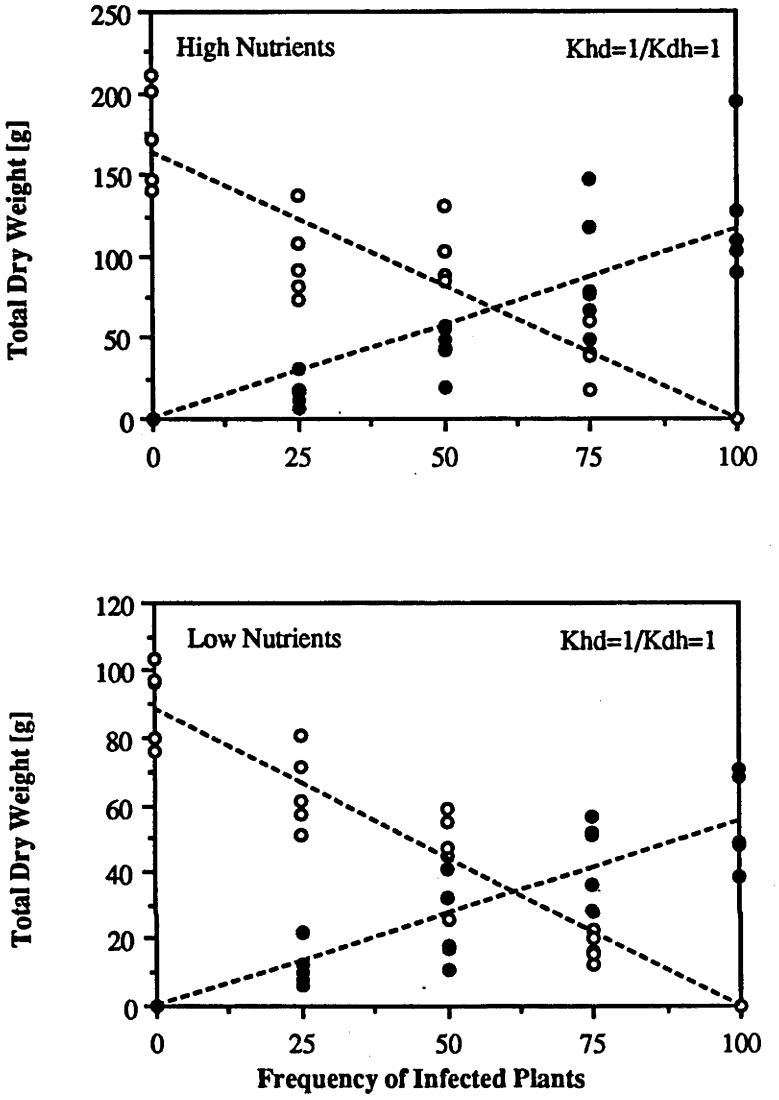


Fig. 11. Replacement series for competition between healthy and infected *C. dactylon* plants growing at two nutrient levels. k_{hd} is the relative crowding coefficient. The dashed lines show the fitted relationship $k_{hd} = 1/k_{dh} = 1$; open circles = individual replicate values, healthy plants; closed circles = individual replicate values, infected plants.

Root / shoot ratio

There was a marked difference in the magnitude of equivalent R/S ratios between Experiments I and II, although a similar overall pattern was expressed. Thus the R/S ratio of *C. dactylon* was significantly affected by smut ($P < .001$) and high nutrient levels ($P < 0.0001$), but not by the frequency of infected plants in the pot ($P > 0.05$). The interactions frequency x nutrients and nutrients x disease status were not significant ($P > 0.05$). At high nutrient levels, healthy plants had higher R/S ratios than infected individuals, except in the 25:75 mixture where that of the infected plants was higher. In contrast in all mixtures and monoculture at low nutrient levels, the R/S ratio of healthy individuals was higher than that of the infected ones (Fig. 12).

Survival

Survival was not significantly affected by disease or the level of nutrients ($P > 0.05$). A low mortality (4%) was detected among infected individuals growing at high nutrient levels.

DISCUSSION

The effect of *U. cynodontis* on its host *C. dactylon* is variable. In addition to its total destruction of seed production, it reduces overall dry matter production, the growth rate of stolons and the survival of *C. dactylon* plants under crowded conditions. However, this systemic smut did not affect the germination of seeds, the emergence of seedlings, or the competitive ability of the plants.

U. cynodontis reduced the dry weight of infected individuals growing in monoculture and mixture regardless of plant density (Fig. 7 and 11). These effects were complicated by interactions between plant density and soil nutrient level. Thus disease and the nutrient levels in the soil were important in determining the outcome of infection. Disease consistently reduced the dry weight of *Cynodon* plants growing in soil with a high level of nutrients, but showed differing effects in low nutrient soil depending on stand density (Figs. 8 and 11). Similar patterns have been reported for *Senecio vulgaris* infected by the non-systemic rust *Puccinia lagenophorae* (Paul and Ayres 1986b). Furthermore, Wennström and Ericson (1994) found that biomass of *Lactuca sibirica* clones infected by the systemic rust *Puccinia minussensis* was significantly reduced in

most of the clones when grew in high nutrient conditions, while only few clones growing in poor conditions showed reduction in biomass.

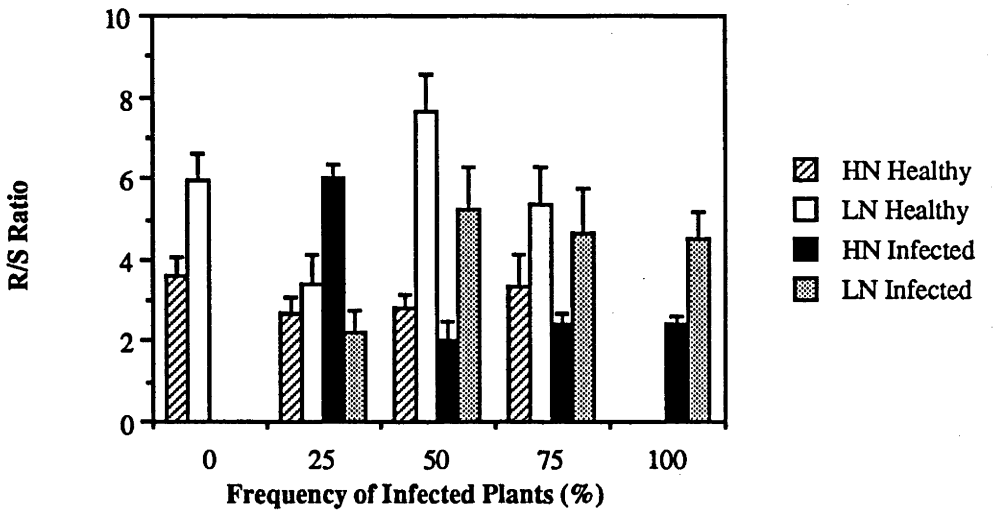


Fig. 12. Root/shoot ratio for healthy and infected *C. dactylon* plants growing at high (HN) and low (LN) nutrient levels and five frequencies of infection. Vertical bars represent ± 1 SE.

In spite of the negative effect that *U. cynodontis* infection had on growth, experiment II showed that these reductions did not affect the competitive ability of infected plants, even under the increased environmental stress caused by low nutrient levels. Both healthy and infected individuals competed equally for the same limiting resources ($k_{hd} = 1/k_{dh} = 1$; Fig. 11). As discussed in Chapter 2, this lack of effect of the disease on the competitive ability of individuals evidenced by even when competition is clearly occurring: cf.

declining plant size with increasing density is quite different to that observed for non-systemic rusts and mildew diseases (Burdon *et al.* 1981; Burdon and Chilvers 1977). Systemic diseases of clonal plants seem to affect the competitive ability in different ways, and it has been suggested that growth patterns may be of importance in explaining this variation in responses. Wennström (1994), for example gives some evidence to show that systemic infection of clonal plants with strong lateral growth appears to have a negative effect on the competitive ability of the host plants. In contrast, in clonal plants with weak lateral growth disease may increase the competitive ability of the hosts (eg. *Puccinia pratensis* infecting *Pulsatilla pratensis*; Wennström and Ericson 1991; Wennström 1994), or have little or no effect (eg. *Microbotryum violaceum* infecting *Silene dioica*; Carlsson and Elmqvist 1992).

In a plant such as *C. dactylon*, disease can potentially affect not only overall growth but also germination and stolon extension. The results of the present study showed that smut disease did not significantly affect proportion and time of emergence of seedlings. A previous study with the clumped grass *Bromus catharticus* (cf. Chapter 2) showed that infection by the smut fungus *Ustilago bullata* had little effect on the emergence of seedlings. It is possible that pathogens such as these smuts, that infect the seed as soon as it starts to germinate, and rely on successful seedling establishment for their own survival will tend to have little or no effect on host seed germination. There may be strong selection against an early damaging effect. In contrast, in ovule infecting smuts, such as Karnal bunt of wheat, *Neovossia indica,* germination of seeds can be significantly reduced by severe infection (Singh and Krishna 1982).

It has been suggested that systemic fungi (pathogens and endophytes) may be important selective agents for growth patterns of clonal plants. Fungal pathogens may favour lateral growth or fragmentation, so that host plants can escape from disease. In contrast, mutualistic associations, in particular those involving endophytic fungi may favour slow growth and delayed fragmentation, so that spread of the endophyte within the host tissues is enhanced (Wennström and Ericson 1992). Thus for *C. dactylon* it is possible that selection has favoured some mechanisms to escape from infection by *U. cynodontis*. Strong lateral growth has been shown to enable host plants to escape from systemic diseases (Wennström 1994). Such effects seem to be common among dicotyledonous clonal plants, for example *Lactuca sibirica* infected by the systemic rust *Puccinia minussensis* (Wennström and Ericson 1992), *Trientalis europaea*

infected by the systemic smut *Urocystis trientalis* (Wennström and Ericson 1990), or *Cirsium arvense* infected by the systemic rust *Puccinia punctiformis* (Frantzen 1994).

However, information on the effect of smut-diseases on the growth of stolons and disease transmission in clonal grasses is very limited. There is some evidence of decreased rhizome growth in *Poa pratensis*, a rhizomatous grass species, when infected by the systemic smut *Urocystis agropyri*, but growth is not affected by the systemic smut *Ustilago striiformis* (Nus 1990). In the current study I found that infection by *U. cynodontis* smut reduced the growth of *Cynodon* stolons (Fig. 4). However, though the smut did spread along the longest stolons there was a tendency for sections near the tip to remain healthy when separated (Fig. 5 and 6).

Disease induced changes in resource allocation patterns may have significant consequences for the performance of infected individuals. Here I found that *U. cynodontis* appears to have an impact on the performance of *C. dactylon* by affecting the development of the root system (Fig. 9, 10 and 12). The effects of *U. cynodontis* on R/S ratios were variable, but there was a general tendency towards reduced R/S dry weight ratios for infected individuals in both experiments. In Experiment I, at low nutrient levels healthy and infected plants from monocultures had very similar R/S dry weight ratios, but in mixtures they were considerably smaller for infected individuals (Fig. 10). Similarly, in experiment II infected plants growing at low nutrient levels had smaller R/S ratios than the healthy individuals (Fig. 12). Possibly the roots are responding more to competition than the shoots, but variation in responses as well as the marked difference in the magnitude of equivalent R/S ratios between experiments I and II cannot be explained.

For other associations involving either, systemic or non-systemic pathogens, infection commonly results in reduction of the root system, for example *Senecio vulgaris* infected by the non-systemic rust *Puccinia lagenophorae* (Paul and Ayres 1986c), *Chondrilla juncea* infected by the non-systemic rust *Puccinia chondrillina* (Burdon *et al.* 1981) and *Bromus catharticus* infected by the smut fungus *Ustilago bullata* (cf. Chapter 2). Reduced root to shoot ratios may result in reduced drought tolerance, reduced ability to recover from shoot damage such as grazing or reduced ability to respond to nutrient supply in infected plants; such conditions might critically reduce the abilities of the infected plants to

compete with individuals of the same or other species particularly when grown in poor environments. Indeed, in my experiments *U. cynodontis* had a negative effect on the survival of *C. dactylon* when grew at high densities and/or at low nutrient levels. This suggests that in crowded and poor environments infected plants with poor root systems are more vulnerable than their healthy counterparts.

This study shows that *U. cynodontis* has traits that may both positively and negatively affect the performance of *C. dactylon*. The total sterilization of the host, indicates that infected *C. dactylon* plants have to rely on vegetative growth to reproduce, and as I have shown healthy individuals can be derived from infected plants. Whether this occurs under natural circumstances is unknown. This possible tendency to escape from disease, as well as the negative effect on the growth of stolons which enable exploitation of favourable sites, also suggest that the association between *U. cynodontis* and *C. dactylon* could be classified as pathogenic. In contrast the unexpected lack of effect on the competitive ability as well as in the emergence of seedlings could indicate a more subtle association. However, smut disease in this species of grass might be of major importance under high densities and poor nutrient conditions, where reduced survival in diseased hosts might affect the size and structure of populations by the creation of vacant niches available for colonization by healthy plants of the same species or other non-host plants.

SECTION B

INCIDENCE AND EFFECT OF SMUT DISEASE IN NATURAL POPULATIONS

This section comprises three chapters related to the association between the flower-smut fungus *Sporisorium amphilophis* and its host *Bothriochloa macra.* Chapter 4 examines regional (among populations) and local (within populations) variation in smut disease incidence as well as the effects of smut infection on growth, survival and competitiveness of *B. macra.* Chapter 5 investigates possible mechanisms of infection of *B. macra* by the systemic flower-smut *S. amphilophis* Chapter 6 explores the possible role of a Phalacrid beetle, in the dynamics of smut infection on *B. macra.*

CHAPTER 4

Regional and local patterns in the spatial distribution of the flower-infecting smut fungus *Sporisorium amphilophis* (Syd.) Langdon & Fullerton in natural populations of its host *Bothriochloa macra* (Steud) S.T. Blake

INTRODUCTION

Host-pathogen associations typically show considerable spatial variability at all levels of scales ranging from the relative occurrence of pathogens on adjacent host plants within individual populations through to the occurrence of populations on a species-wide scale of distribution (Burdon 1993). Determining the causes of this variability and its degree of permanence is essential to an understanding of the ecological and evolutionary role that pathogens play in any particular association.

Ultimately, the severity of disease in any population, and hence its evolutionary consequences, is determined by the combined effects of aspects of the abiotic and biotic environment. On a broad geographic scale, the abiotic environment is typically the major determinant of the distribution of disease. Even when resistance in the host is shown to have marked regional distributions (eg. resistance to *Puccinia coronata* in *Avena* spp., Dinoor 1970) this pattern is related to underlying differences in climatic variables like temperature and rainfall which ensure that some areas are inherently more favourable for disease development than others. Over time such increased disease activity has favoured host individuals carrying resistance genes. In contrast, at the level of the population both abiotic and biotic factors play a role in determining the incidence and severity of disease on individual host plants. Certainly at this scale microsite differences in degree of exposure may influence disease levels (Jarosz and Burdon 1988; Dinoor and Eshed 1990) but biotic factors which have no role to play at higher spatial scales may also have a substantial effect. Thus biotic variables like host density or the presence of other competing, non-host species may substantially affect the incidence and severity of disease.

Understanding the ways in which the interplay of abiotic and biotic factors may affect the incidence of disease within and among host populations and the geographic scale of these effects is of considerable importance in determining the physical boundaries of host-pathogen metapopulations. To date most studies that have documented systematic geographic changes in either disease incidence or resistance have examined changes over hundreds or thousands of kilometres (Dinoor 1970; Burdon *et al.* 1983) and clearly may encompass more than one metapopulation (Burdon *et al.* 1983). Here I am interested in examining the interaction of biotic and abiotic effects involving the incidence of a systemic flower-infecting smut (*Sporisorium amphilophis*) in natural populations of its grass host *Bothriochloa macra* over a much shorter distance.

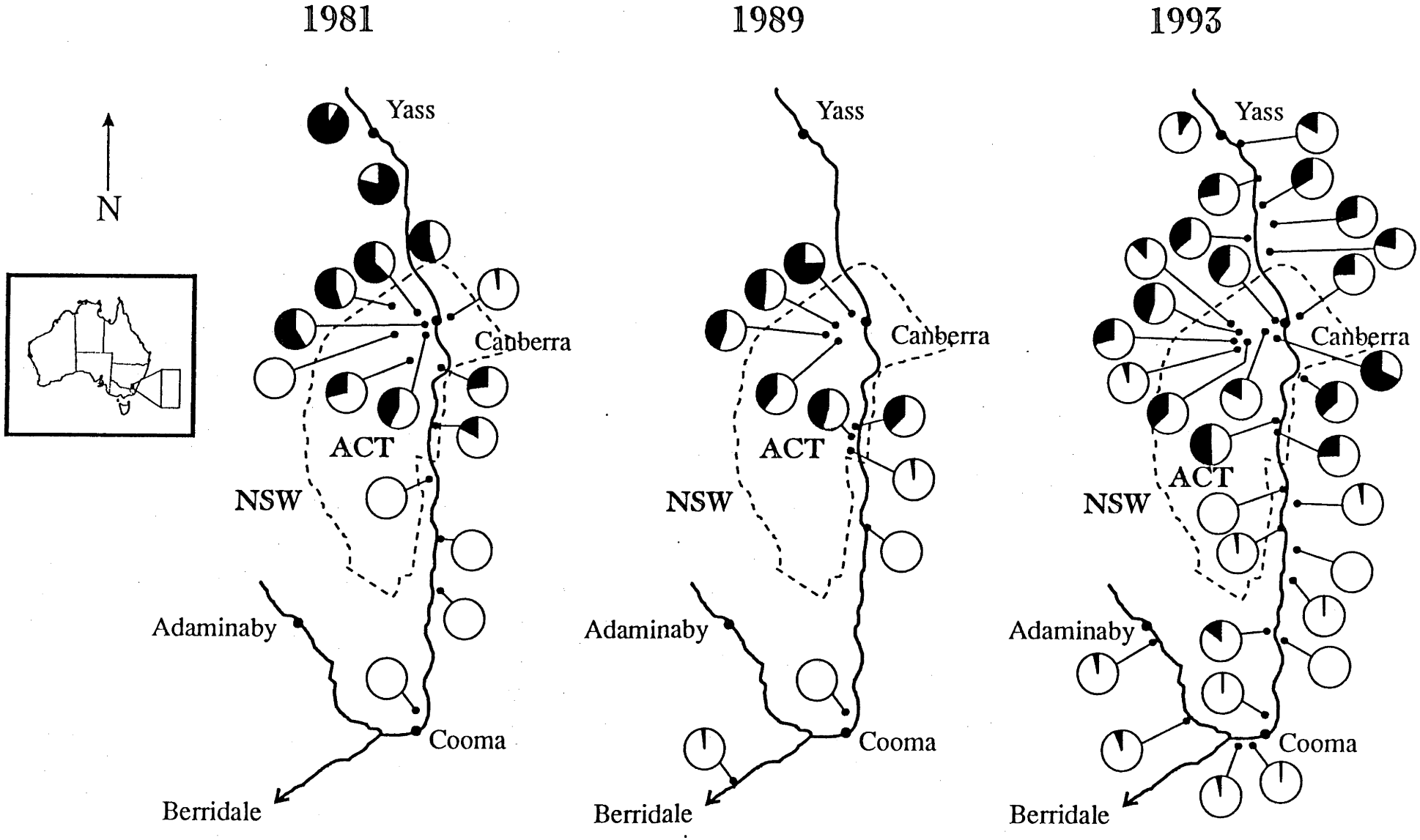
In 1981 a preliminary 250 km transect survey uncovered a marked transition zone with the frequency of plants infected by this smut changing rapidly over the space of only a few kilometres. My study focuses then on two sets of questions. The first of these concern: (i) the temporal consistency of the pattern of smut distribution observed in 1981; and (ii) the cause of this pattern. In particular, I aimed to determine whether the observed pattern was simply a static snapshot of an invasive process or whether abiotic aspects of the environment provide a plausible explanation. The second set of questions focus on the interaction between host and pathogen at the level of the individual population and aims to determine whether biotic forces really do influence local smut frequencies. I do this through the measurement and correlation of potential factors (density, presence of other species) in the field and through glasshouse competition experiments.

METHODS

Host-pathogen association

Bothriochloa macra is a native tufted perennial grass that is found in grasslands and woodlands, usually growing on loams and clays in eastern Australia. This grass often survives in over-grazed native pasture and is frequently found colonising disturbed areas. Established plants increase in size by tillering (Lamp *et al.* 1990). The main flowering time for *B. macra* is early summer, although inflorescences can be found from November to April. Most seeds are shed in April. Germination occurs until late spring (November) when soil

Fig. 1. Geographic distribution of 16, 10 and 31 populations of *B. macra* sampled in 1981, 1989 and 1993 respectively, with the percentage of smut-infection for each population shown by black portion of pie diagram.


1981
1989
1993
N
Yass
Canberra
ACT
NSW
Adaminaby
Cooma
Berridale

temperatures are high enough to enable rapid germination and seedling establishment (Hagon 1976).

In the field *B. macra* is often infected by the floral-smut fungus *Sporisorium amphilophis*. This pathogen affects plant fitness by replacing all reproductive structures with a spore mass. Once infected, plants remain diseased for their entire life.

The mechanism of infection of *B. macra* by *S. amphilophis* is unknown, but previous work on the infection processes and life cycle of other floral smuts attacking grasses, indicate that there are two distinct methods. The commonest of these involves infection of the germinating seedling by spores in the soil or carried on the seed coat (for example, *Ustilago bullata* infecting *Bromus* species). Other studies (Chapter 4) show that this is not the method of infection employed by *S. amphilophis*. The second possible method involves embryo infection with entry occurring in the stigma at anthesis (for example *Tolyposporium penicellariae* infecting pearl millet, Thakur 1989, and *Ustilago nuda* and *Ustilago tritici* attacking barley and wheat respectively, Nielsen 1988; Wheeler 1968). In this case the mycelium develops in the ovary and thereafter in the dormant seed. We have yet to demonstrate this form of infection in the *S. amphilophis* - *B. macra* interaction. Once germination and development of the host plant occur, the mycelium resulting from either form of infection grows throughout the tissues of the seedling and eventually sporulates in the flowering stage, when the spores are again formed (Agrios 1978).

Regional patterns of disease incidence

Surveys of the incidence of *S. amphilophis* infection of *Bothriochloa macra*, were carried out in a series of *B. macra* populations located in a north-south transect of ca. 230 km, from Bowning, NSW (34°46' S, 149°49' E) through the Australian Capital Territory and adjacent region of New South Wales to the edge of the Kosciusko Natural Park (35°56' S, 148°39' E; Fig. 1). Surveys were carried out in 1981, 1989 and 1993 and assessed 16, 10 and 31 populations respectively. During this period many of the 1981 populations were destroyed by urban expansion, road reconstruction and or changes in farming practices. Additional populations were added to compensate for these losses and to extend the geographic range of observations. Over the transect the altitude ranges from 550 to 1300 m; total yearly rainfall ranges from 472 to 1132 mm; and mean

monthly temperatures range from 27°C to 9°C during the summer and from 14°C to -0.2°C in the winter.

In each population, 100 *B. macra* plants were selected haphazardly with the restriction that all samples were at least 1 m apart from each other and that all areas of the population were sampled. Plants were selected by walking through the population throwing a plastic marker and scoring the plant found closest to the point. For each plant, the disease status (healthy or diseased), average height of inflorescences, total number of inflorescences and number of healthy and infected inflorescences were recorded. The accuracy of this method in assessing disease status was confirmed in two ways. First, preliminary surveys of isolated infected plants indicated that all inflorescences produced by individual infected plants showed typical disease symptoms (Fig. 2). Usually the entire inflorescence was smutted, however, occasionally an inflorescence would be only partially transformed. Second, tillers of *B. macra* known to be infected showed smutted inflorescences through 2 cycles of flowering in the glasshouse. Control, uninfected plants always produced healthy inflorescences.

Temperature and rainfall are two of the principal physical factors that affect the distribution of plant diseases (Burdon 1987). As a consequence, for each host population aspects of these factors were estimated using the computer program ESOCLIM. This program was developed at the Center for Resource and Environmental Studies (CRES), Australian National University and uses methods developed by Hutchinson (1989, 1991) to estimate climate data at user defined locations from "thin plate smoothing spline" coefficients (J. McMahon pers. comm.). Here we estimated mean maximum, minimum temperatures and rainfall for each season of the year and the number of frost days.

Data analysis: Relationships between levels of smut disease in host populations and climatic variables were analysed using logistic regression (McCullagh and Nelder 1989). The response variable considered in the model was the proportion of infected plants per population. The independent variables were seasonal minimum and maximum temperatures and number of days with frost during late autumn (May), winter (June to August) and early spring (September).

Variation in height and number of inflorescences of *B. macra* plants was separately analysed using multiple linear regression, with the independent variables population, plant condition (healthy or infected) and their interaction.

Fig. 2. The effects of infection by the floral-smut fungus *Sporisorium amphilophis* on *Bothriochloa macra*. Left: smutted heads; right: healthy inflorescences.

Local spatial patterns and the effect of smut-disease on plant fitness

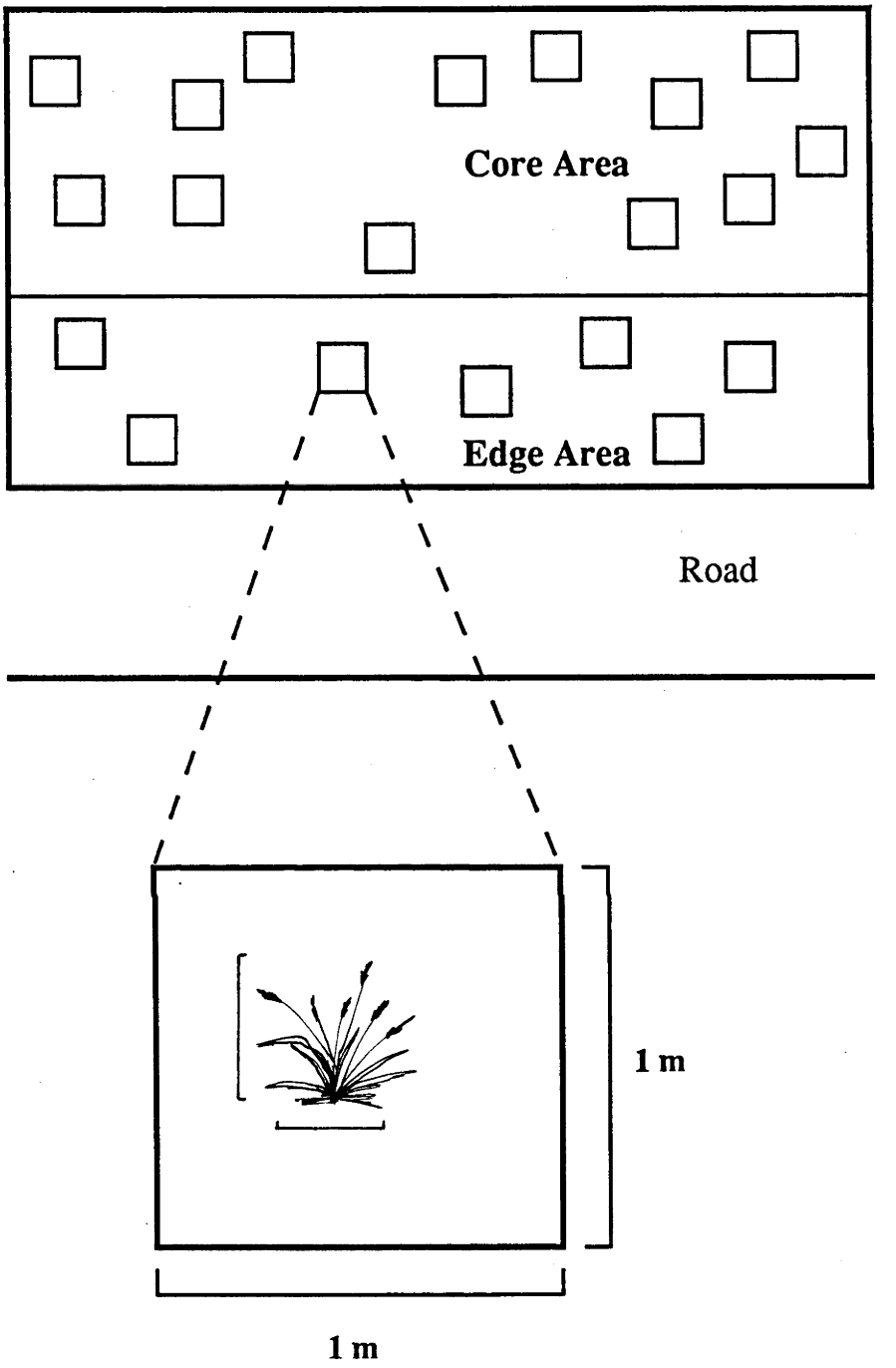
Spatial patterns of disease occurrence in relation to the density and spatial heterogeneity of local plant populations were determined in field surveys. These were carried out during the summer of 1993 (March and April) in five *B. macra* populations located in the Australian Capital Territory area. The percentage of plants infected in these populations ranged from 13 to 40%.

Preliminary observations suggested that the percentage of infection might be higher towards the edge of *B. macra* populations. To test for this possibility, each population was divided in two areas: edge and core (Fig. 3). Edge areas were obviously disturbed, with less than 40% ground cover and occurred up to 5-10 m from the roadside. Core areas had ground cover of 60 to 100% with little or no disturbance. In each area, twenty 1 m^2 quadrats were placed at random, and all *B. macra* plants within these recorded. In addition, the number of other herbaceous plants present in the quadrat was scored. For each *B. macra* plant, disease status (healthy or infected), basal diameter, height of the inflorescences, number of inflorescences, and number of infected and healthy inflorescences were recorded.

Data analysis: The factors which may affect local spatial patterns of smut-diseased plants were analysed using Poisson regression modelling (McCullagh and Nelder 1989). The response variate was the number of infected plants per quadrat. The error distribution for the counts was stipulated to be Poisson. Variation in this variate were modelled in terms of population, area (edge versus core), logarithm of density (number of *B. macra* plants per m^2), and number of dicotyledonous and monocotyledonous plants per quadrat.

For studying variation in diameter, height and number of inflorescences of *B. macra* plants, an unbalanced linear mixed-analysis model was used and so restricted maximum likelihood estimation (REML) was required (Engel 1990). The number of inflorescences was square-root transformed prior to analysis. The independent variables were disease status (healthy or infected), area (edge versus core) and density within population.

Fig. 3. Technique used to assess spatial patterns of smut disease occurrence in relation to spatial heterogeneity of local *B. macra* populations.


Core Area
Edge Area
Road
1 m
1 m

Effect of *S. amphilophis* on the competitive ability of *B. macra*

To assess the effect of smut disease on the competitive ability of *B. macra,* infected and healthy plants were collected from the field and divided into tillers. The tillers were planted in flats and kept in a naturally-lit glasshouse until established. Once established, they were planted at three different densities (4, 8 and 16 plants per pot) and five percentages of infection (0, 25, 50, 75 and 100% infected plants per pot) in 15 cm pots filled with standard potting mix. Each treatment was replicated five times. An additional experiment examining the effect of nutrient level on the performance of this host-pathogen interaction was carried out at the same time by repeating the 16 plants per pot treatment in a lower nutrient soil mix (75% sand: 25% potting mix). All pots were kept in a naturally-lit glasshouse and watered as necessary. Plants were harvested after 4 months at which time all plants were flowering (from this I confirmed that all infected tillers were indeed diseased). The roots of harvested plants were washed and all plants dried at 60°C for one week. Shoot and root weights were recorded separately for each individual and the number of dead plants per pot was scored.

Data analysis: Description of the effects of *S. amphilophis* on the competitive performance of *B. macra* was achieved by using maximum-likelihood estimates for the parameters of the non-linear de Wit competition model (Machin and Sanderson 1977) to determine values for k (the relative crowding coefficient). Values of k give a quantitative measure of the relative competitiveness of two species: k values greater than unity imply the species concerned is more aggressive than the species with k values less than unity. The model initially assumes that the two species are not competing for the same resources by fitting the data to the $k_{hd} \neq 1/k_{dh}$ submodel. It then fits the data to the second submodel, that is $k_{hd}=1/k_{dh}$ (the two species are competing for the same resources but are unequal in their relative competitive ability). If the second submodel is not a significantly better fit, then the first , ie. $k_{hd} \neq 1/k_{dh}$ is the model accepted. However, if there is a significant difference, a third submodel is fitted to the data - that $k_{hd}=1/k_{dh}=1$. In this circumstance the two species are competing for the same resources and are equally competitive. Again if the third submodel is not significantly better, the second submodel is accepted. This sequential process of model fitting progressively reduces the number of parameters estimated as it works from the maximal to the most constrained model.

To determine the effect of density, smut-disease, the proportion of infected plants and nutrient levels on the root/shoot (R/S) ratios of *B. macra* plants, data were analysed using an unbalanced linear mixed model; a restricted maximum likelihood estimation (REML) was required (Engel 1990). Root/shoot ratios were log transformed prior to the analysis. Survival was analysed using logistic regression (McCullagh and Nelder 1989). The fitted terms considered were density, proportion plants infected per pot, disease status and nutrient levels.

RESULTS

Regional patterns of disease incidence

The overall pattern of incidence of infection by *S. amphilophis* was very similar for the three survey times (Fig. 1). In all years there was a general trend towards higher levels of infection in the more northerly populations and those located in the Australian Capital Territory and lower levels in populations located to the south. The abrupt nature of the geographic shift from infection levels averaging more than 25% to those averaging less than 5% is clearly shown in the most extensive data set (that for 1993; Fig. 4). In addition, however the number of southern populations in which at least some infected individuals were present increased over time.

Detailed statistical analysis of patterns in the spatial distribution of smut infection are restricted to the extensive 1993 data. In this data set the percentage of infection for each population ranged from 0 (three sites) to 68% (one site; Fig. 4). A logistic regression showed that levels of infection were significantly related to temperature, but especially to the number of days with frost during winter. In particular, the analysis indicated that populations which had greater number of days with frost during July (mid-winter) had significantly smaller numbers of infected plants ($P < 0.003$; Fig. 5). No significant relationships occurred between disease levels in populations and either mean seasonal temperature or rainfall, once days with frost had been included in the model.

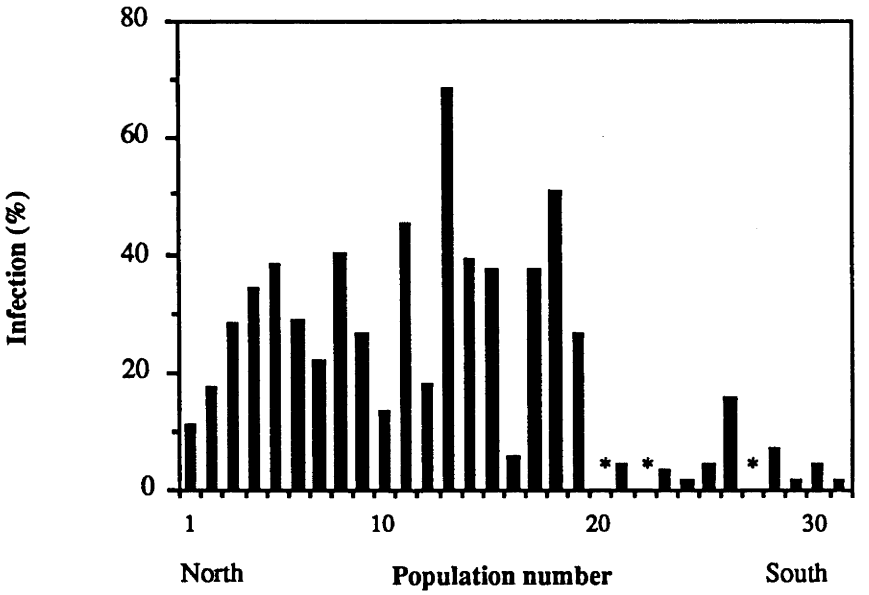


Fig. 4. Geographic variation in the percentage of infection for the 31 populations of *B. macra* surveyed in 1993. These are arranged in order from north to south. * Zero infection for populations 20, 23 and 26.

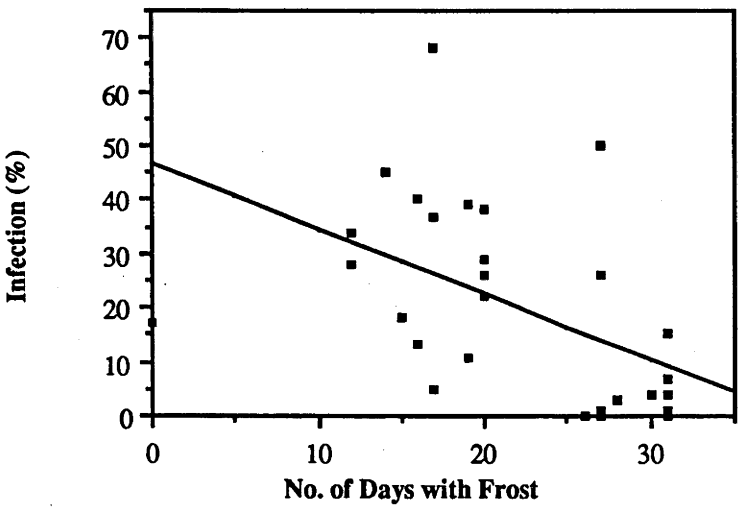


Fig. 5. Relationship between number of days with frost in July and the proportion of infected *B. macra* plants ($y = 46.50 - 1.20x$, $r^2 = 0.26$, $P < 0.003$).

Across all populations healthy plants tended to be taller than infected plants. The height of healthy inflorescences ranged from 42 cm to 60 cm and that of infected ones from 23 cm to 58 cm (Fig. 6A). Statistical analysis of the logarithm of height, showed disease status, population and their interaction had significant influences on the height of plants ($P < 0.001$ for disease status and population, $P < 0.001$ for population x disease status). The interaction term appears to relate to the generally greater reduction in height of the southern populations.

Infected plants tended to produce more inflorescences (2 -13) than healthy plants (3-6; Fig. 6B). Multiple regression analysis of the logarithm of the number of inflorescences, revealed significant influences of disease status and population ($P < 0.001$). The number of inflorescences declined from north to south.

Comparisons of the incidence of disease in four populations surveyed on all three occasions (Fig. 7) showed some variation in the levels of infection with time. In one population (Black Mountain) the highest levels of infection were recorded during 1989, but in the last survey a significant reduction in the levels of infection occurred. In the Coppins Crossing population the highest levels of infection were found in 1981 and a lower percentage of infected plants during 1993. In contrast with the two earlier surveys (1981, 1989) when infection was absent, the survey of 1993 detected a very small percentage of infected plants in the population of Michelago. Infected plants were never found in the Bunyan population.

Local spatial patterns in disease incidence

Levels of infection vary from population to population and between areas within the five Canberra populations studied in detail (Fig. 8). The highest percentages of infection were found at Black Mt. and Coppins Crossing populations, the lowest at Uriarra Rd. The proportions of plants infected at the Barren Joey Dr. and Cotter Rd. sites were similar.

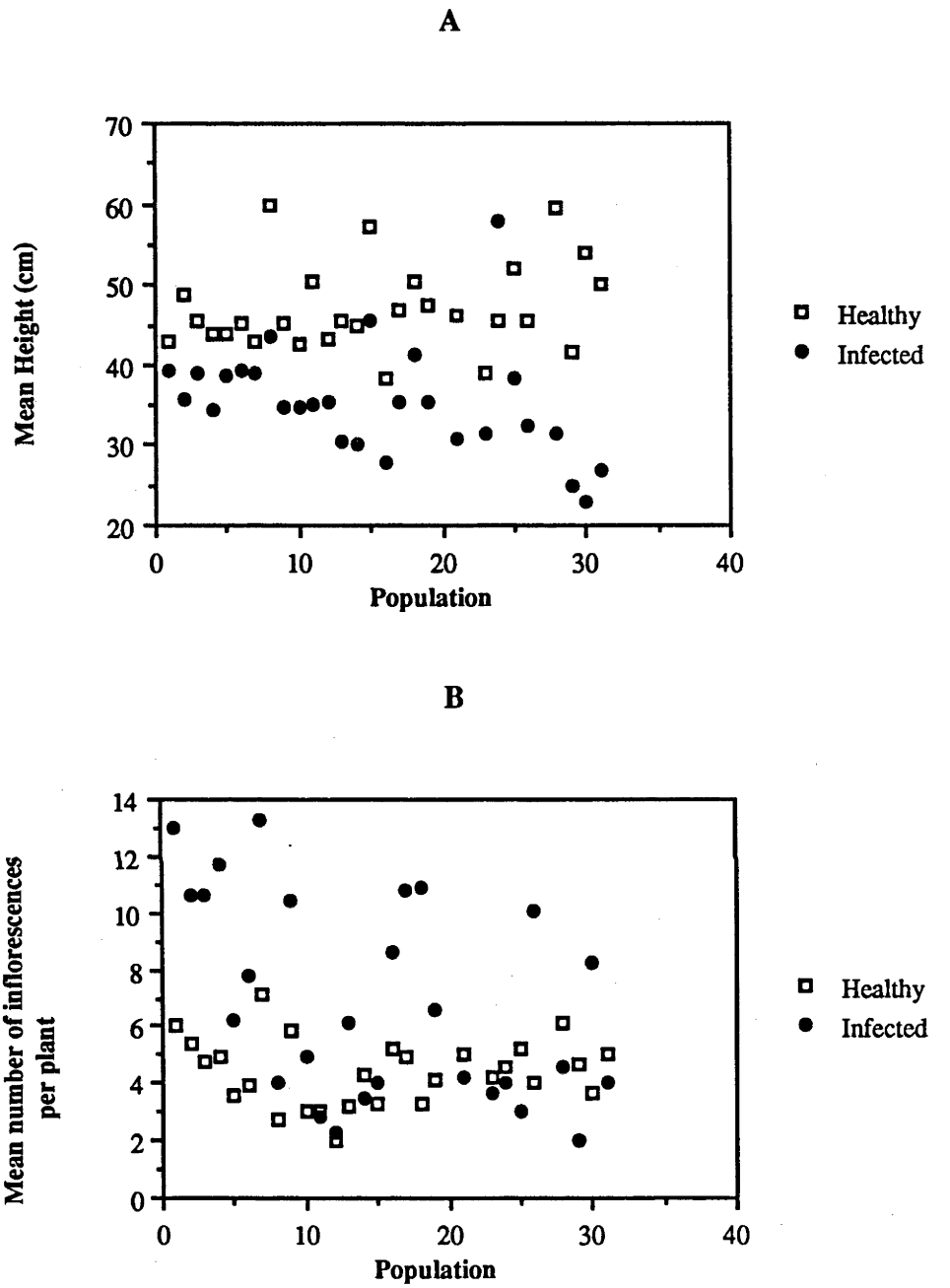


Fig. 6. Height (A) and average number of inflorescences (B) for healthy and infected plants of *B. macra* in 28 populations arranged in order from north to south.

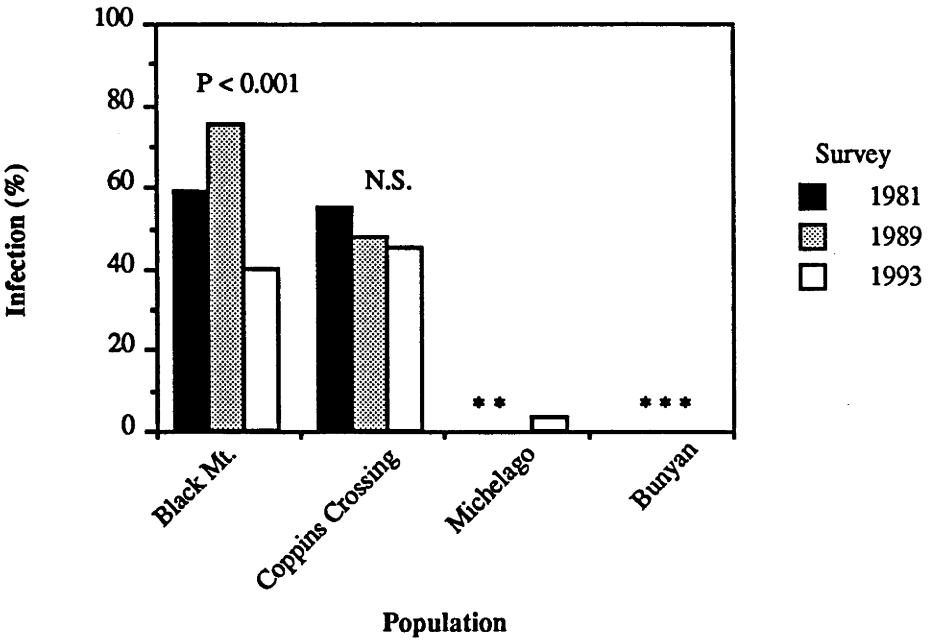


Fig. 7. Temporal variation in the percentage of infection by *S. amphilophis* in four populations of B. *macra*. These are arranged along a north-south axis.* Zero percent infection. Among-years ANOVA comparisons for Black Mt. and Coppins Crossing populations. NS, not significant.

A Poisson regression model showed that the population, logarithm of plant density, and area within populations all contributed significantly to differences in the level of smut infection ($P < 0.001$ in all cases). Plant density and plant population had the greatest effect, each explaining more than 20% of the total deviance. The abundance of other monocotyledonous and dicotyledonous species had no significant effect ($P > 0.05$). Area (core versus edge) had a less pronounced but still noticeable effect with the mean percentage of smut infection in core areas being lower (19.07%) than that in edge areas (43.28%; $P < 0.001$; Fig. 8), except at Uriarra Rd. Interaction terms were all either not or marginally significant, explaining a combined total of 8.6% of the deviance only ($P \approx 0.05$).

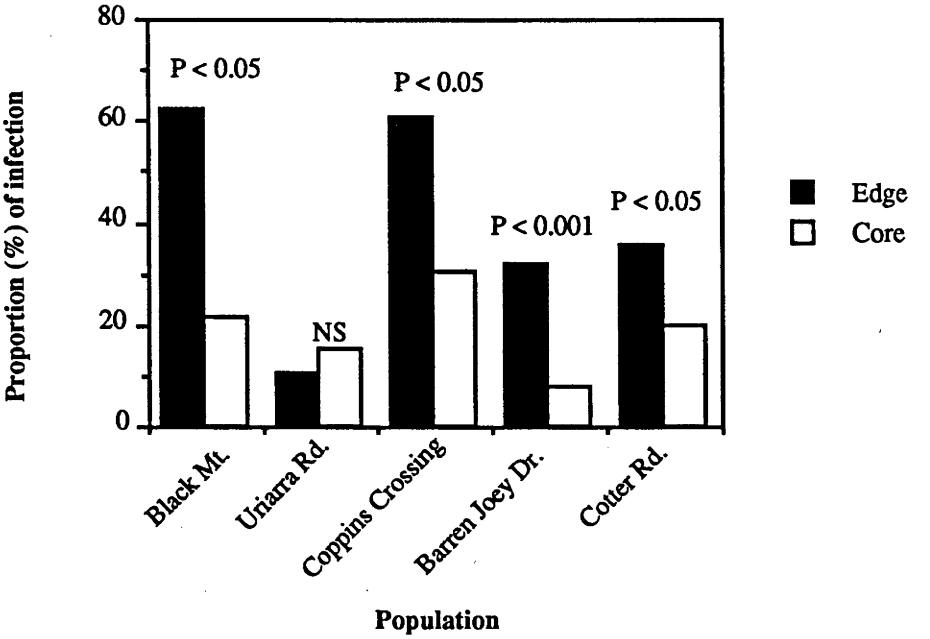
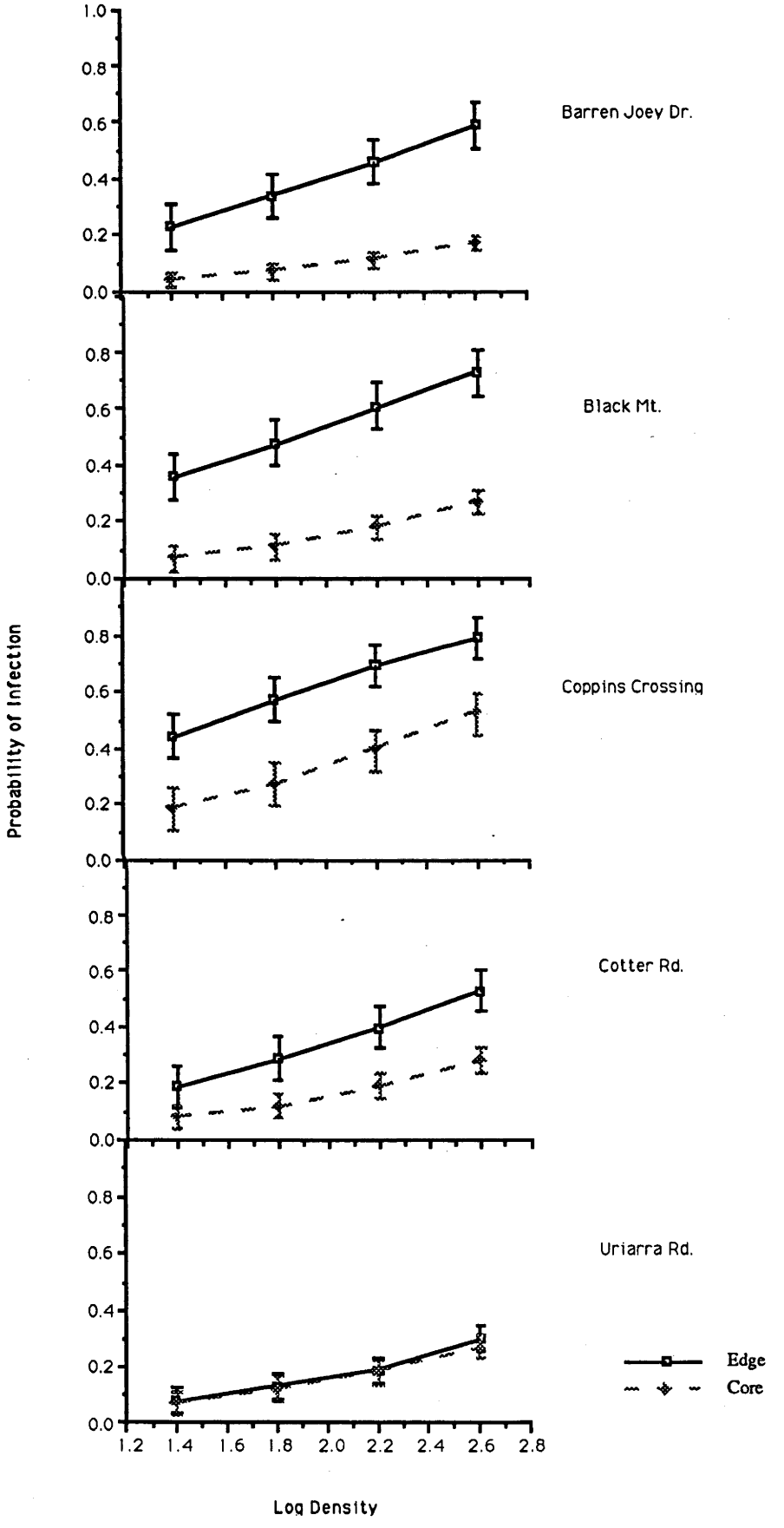


Fig. 8. Proportion of plants infected in core and edge areas of five populations of *B. macra*. Between edge and core areas ANOVA comparison. NS, not significant.

The relative effects of plant density and area (edge or core) on disease incidence was modelled using generalised linear analysis (Fig. 9). In all but one population (Uriarra Rd. site) the probability of infection was conspicuously different between the edge and core areas of a population. For all populations and both edge and core areas the probability of infection increased significantly as density rose (Fig. 9).

Fig. 9. Predicted infection levels plotted against set values of the natural logarithm of density (mean number of *B. macra* plants per m^2) for edge and core areas of the five populations. Vertical bars represent $\pm$ 1 SE.


Barren Joey Dr.
Black Mt.
Coppins Crossing
Cotter Rd.
Uriarra Rd.
Edge
Core
Probability of Infection
Log Density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8

Effect of smut-disease on plant fitness

The most noticeable effect of *S. amphilophis* infection on *B. macra* was the total destruction and replacement of the inflorescences by the pathogen. In addition, infection was negatively related to the height and to the basal diameter of *B. macra* "clumps"and positively to the number of inflorescences (Table1).

On average, inflorescences produced by infected plants were 13.3 cm shorter than those on healthy plants, while inflorescences of plants growing in the edge area were 8.2 cm shorter than those produced by individuals in the core zone. The number of inflorescences was greater on infected plants. On average infected plants produced 4 inflorescences, while the healthy plants produced 3.

Effect of *S. amphilophis* on the competitive ability of *B. macra*

Disease caused by *S. amphilophis* greatly depressed yield of *B. macra* infected plants, so that infected individuals weighed up to 57% less than the healthy ones, in both pure and mixed stands. At higher densities, healthy and infected plants were smaller. The analysis of the complete data set showed that among-replicate variation in the yield of healthy and infected plants was high for all experimental treatments, so that the maximum-likelihood estimates for the parameters of the de Wit competition model showed very large residuals. As a consequence, the data was reanalysed twice after the following restrictions. The first restriction excluded all replicates of mixtures where mortality had occurred, but retained all five replicates from monoculture treatments regardless of mortality levels. An even tighter restriction on the data set excluded all replicates where mortality occurred from the analysis. The competition coefficients (k values) of these analyses are presented in Table 2. From this it is apparent that despite the high residuals encountered in the initial model (probably caused by inconsistency in establishment and growth of tillers) the final interpretation of the model did not differ among the three analyses. The replacement series for the original data set are presented in Fig. 10 and 11.

Quantitative descriptions of the competitive performance of healthy and infected individuals growing under high nutrient conditions indicated that the competition model that provided the best fit was $k_{hd}=1/k_{dh}=1$. This result indicates that despite the yield-depressing effects of smut infection, at all three densities

Table 1. Mean and standard error (SE) of diameter, height and number of inflorescences, for healthy and infected plants, and variation between edge and core areas within five populations studied in detail. Effects of disease and area were tested in a mixed model including populations and the test statistics (Wald test) is shown.

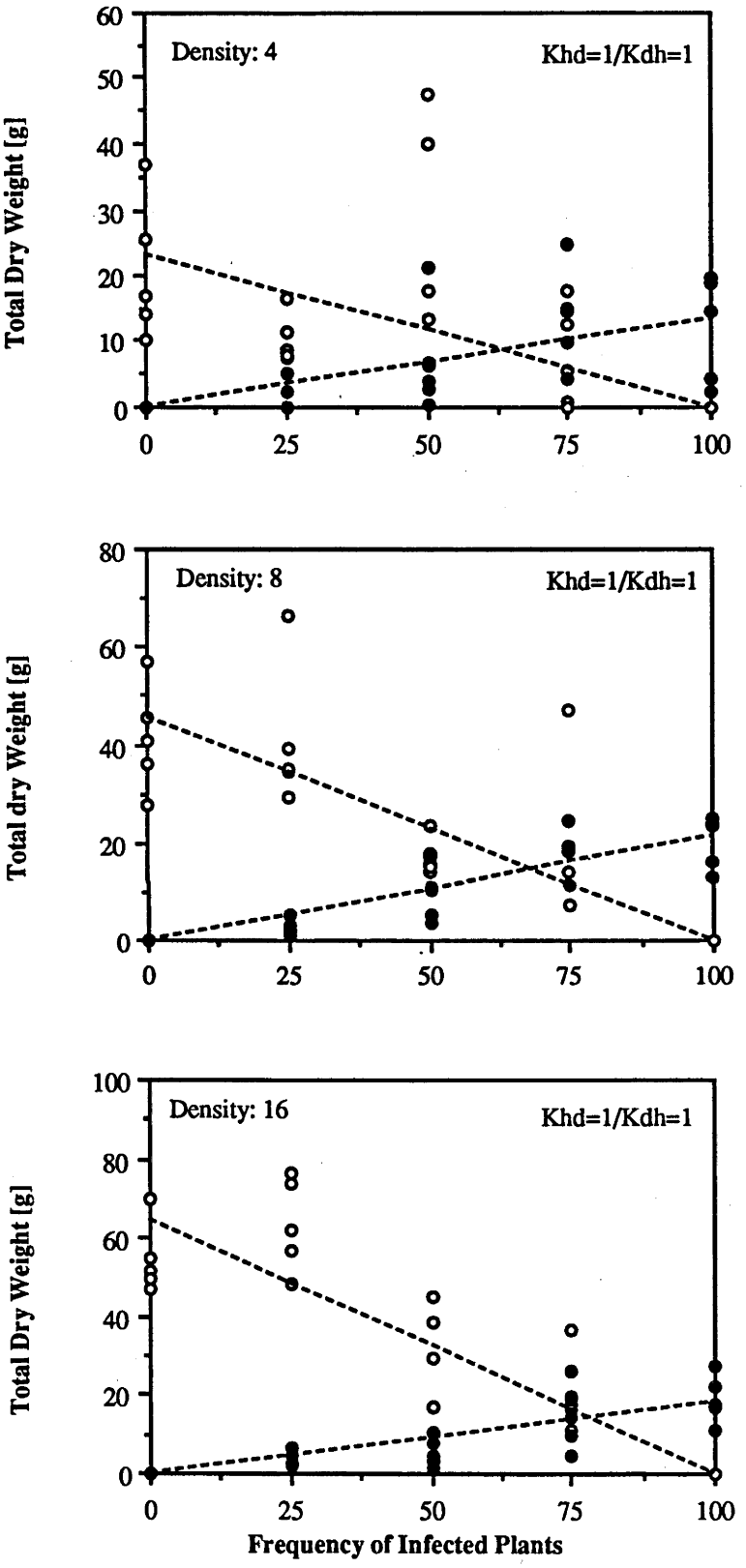
Parameter	Disease Status				Area			
	Healthy X ± SE	Infected X ± SE	Test statistic	P	Edge X ± SE	Core X ± SE	Test statistic	P
Diameter (cm)	9.79 ± 0.38	9.32 ± 0.40	12.51	0.0001	9.55 ± 0.39	10.03 ± 0.41	4.19	0.04
Height (cm)	51.43 ± 3.27	38.12 ± 3.33	391.65	0.0001	48.50 ± 3.39	54.35 ± 3.47	8.06	0.01
Number of inflorescences (Square root transformed)	1.66 ± 0.08	1.83 ± 0.08	26.46	0.0001	1.63 ± 0.08	1.68 ± 0.09	0.41	NS

healthy and infected plants were equally efficient in competing for the same resources (Fig. 10).

Table 2. The fit of the de Wit model to results from the competition experiment between healthy and diseased *B. macra* plants. The complete data set, included five replicates for monocultures and mixtures; restricted set 1 excluded mixture replicates where mortality occurred; and restricted set 2 excluded all replicates where mortality occurred.

	Density [plants/pot] and nutrient level			
Data Set	**4 High nutrients**	**8 High nutrients**	**16 High nutrients**	**16 Low nutrients**
Complete data	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 2.39$; $k_{dh} = 0.42$
Restricted 1	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 2.71$; $k_{dh} = 0.37$
Restricted 2	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 1/k_{dh} = 1$	$k_{hd} = 2.24$; $k_{dh} = 0.45$

Fig. 10. Replacement series for competition between healthy and infected *B. macra* plants growing at three densities. The relative crowding coefficients of healthy and infected plants (k_{hd} and k_{dh}) are presented. The dashed lines show the fitted relationship $k_{hd}=1/k_{dh}=1$; open circles = individual replicate values, healthy plants; closed circles = individual replicate values, infected plants.


Density: 4
Khd=1/Kdh=1
Total Dry Weight [g]
Density: 8
Khd=1/Kdh=1
Total dry Weight [g]
Density: 16
Khd=1/Kdh=1
Total Dry Weight [g]
Frequency of Infected Plants

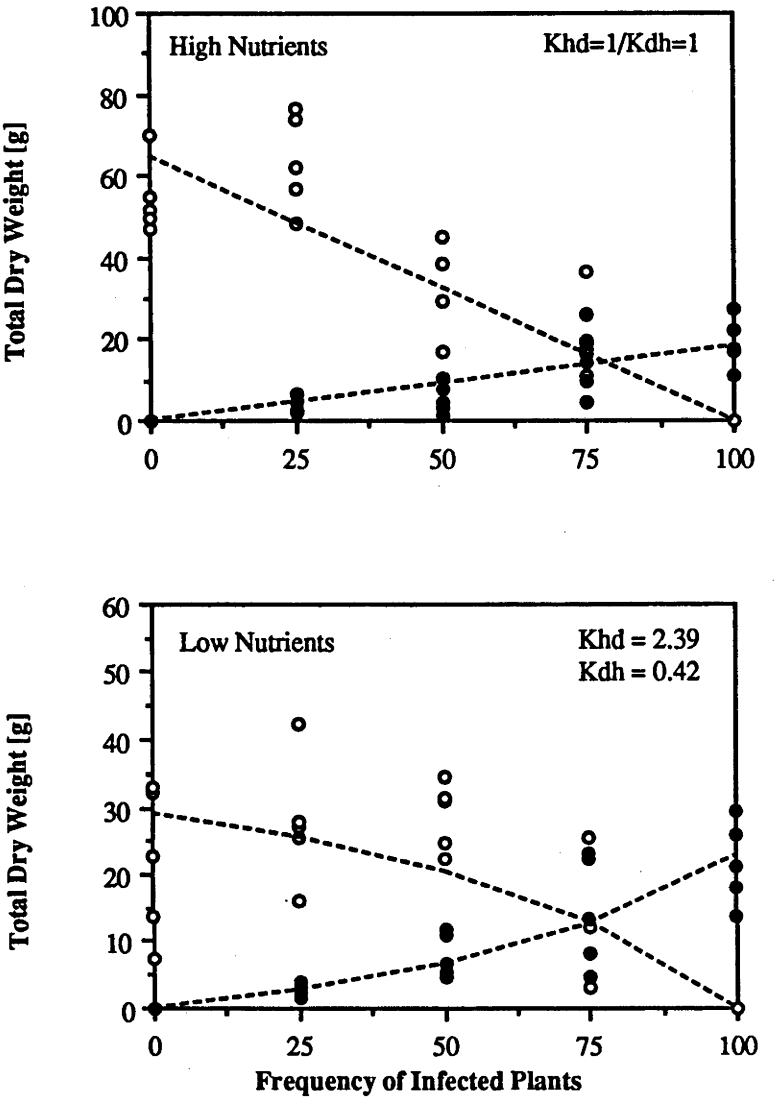


Fig. 11. Replacement series for competition between healthy and infected *B. macra* plants growing at two nutrient levels (density = 16 plants/pot). [The high nutrient results are the same as those shown in Fig. 10]. The relative crowding coefficients (k_{hd} and k_{dh}) are presented. The dashed lines show the fitted relationship k_{hd}=1/k_{dh}=1 (high nutrients) and k_{hd} = 2.39, k_{dh} = 0.42 (low nutrients); open circles = individual replicate values, healthy plants; closed circles = individual replicate values, infected plants.

Effect of the level of nutrients on competition. At low nutrient levels, both healthy and infected plants were smaller than plants growing at high nutrient levels. At high nutrient levels, crowding coefficient indicated that they did not differ from the null model (k_{hd}=1/k_{dh}=1), suggesting that healthy and infected plants competed equally for the same resources. However, in contrast to the results at high nutrient levels, at low nutrient levels the analysis of competitive effects indicated that healthy plants were more aggressive than infected ones while they competed for the same resources (k_{hd} = 2.39, k_{dh} = 0.42; Fig. 11).

Resource allocation

The effect of disease on the allocation of resources within plants was determined by analysing root/shoot (R/S) ratios of all individuals. In general the presence of disease lead to a reduction in the R/S ratio, particularly when plant grew at high densities (Fig. 12; $P < 0.0001$). However, the frequency of infection, the density of plants, the level of nutrients and their interactions had little or no effect ($P > 0.05$).

Survival

In the high nutrient treatment, survival of infected plants was lower (74%) but not significantly so than that of the healthy individuals (87%). In contrast, survival of infected plants growing at low nutrient levels was significantly lower (69%) than that of healthy individuals (84%; $P < 0.04$).

DISCUSSION

Regional variation in disease occurrence

A major problem with most studies of plant-pathogen associations is that they tend to be short-term. The complete understanding of host-pathogen interactions requires knowledge of the levels of variation of disease incidence in time and space. This study contributes in these aspects by showing that the incidence of infection of *Bothriochloa macra* by *Sporisorium amphilophis* over the 12-year period 1981-1993 had a consistent spatial pattern (Fig. 1). In all three surveys, a greater proportion of plants in northerly populations were infected than in populations located to the south. This pattern showed strong positive

relationships with aspects of the abiotic environment such as temperature, and most clearly with low winter temperatures.

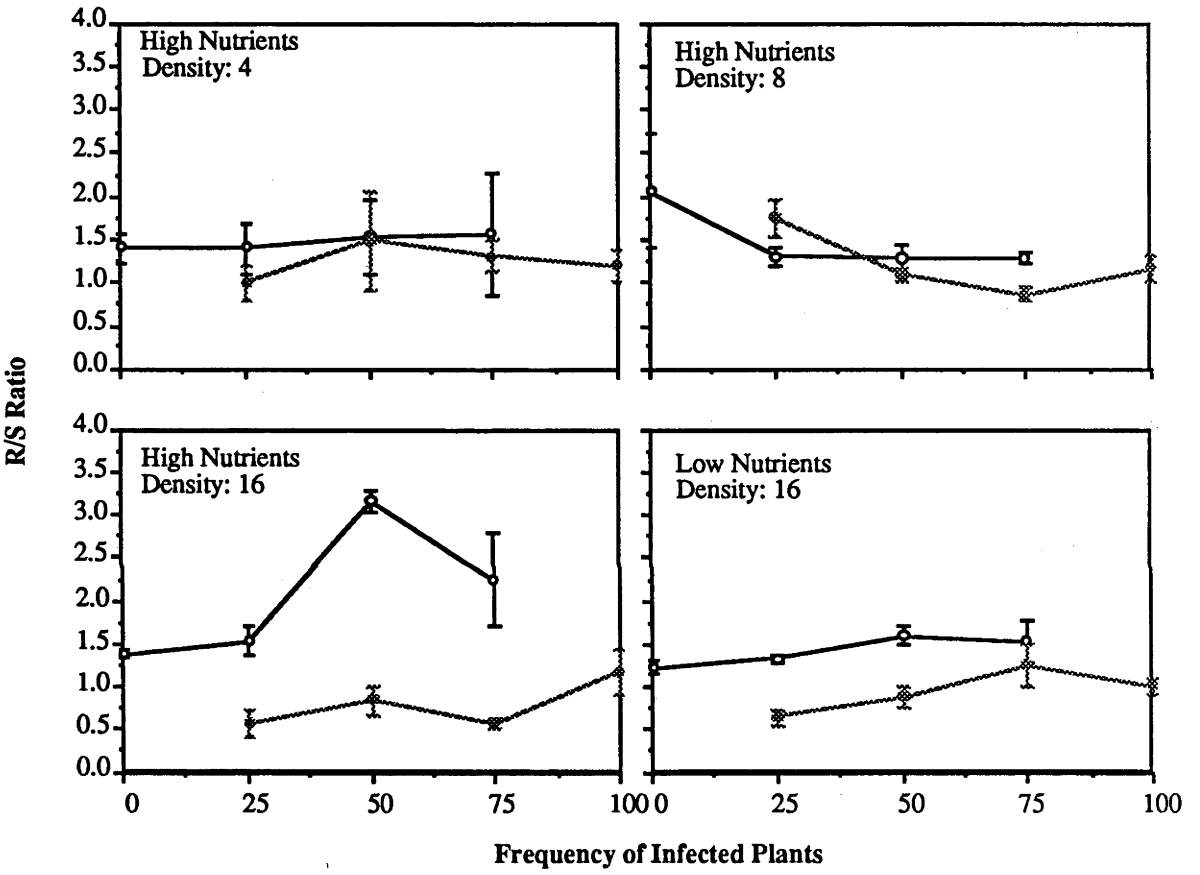


Fig. 12. Root/shoot ratios for healthy and infected *B. macra* plants growing at three densities (4, 8 and 16 plants/pot) , five frequencies of infection and two nutrient levels. Vertical bars represent ± 1 SE. —o— Healthy plants, —•— infected plants.

The abiotic environment is typically the primary limiting factor determining the presence or absence of plant disease. Changes in specific physical factors may affect the pathogen, the host or their interaction by altering: (i) the germination of spores and hyphal growth of individual pathogens; (ii) the germination, growth or susceptibility of host plants; (iii) the expression of disease; or (iv) the survival of infected plants (Agrios 1978; Colhoun 1973).

Currently it is not known how *S. amphilophis* gains entry to its host (see Chapter 5) and hence it is not possible to exclude the possibility that the observed distribution of disease along the transect results from an interaction between the abiotic environment and the infection process. However, the negative relationship between low temperatures and disease incidence may be explained by a greater sensitivity of infected plants to harsh environmental conditions. Increased mortality associated with low winter temperatures has been reported for *Silene alba* infected with *Ustilago violacea* where 31% of infected individuals died over a winter period compared with only 3% of healthy plants (Thrall and Jarosz 1994). Increased mortality of smut-infected plants has also been reported for *Bromus* (Falloon 1976). The reduced R/S ratio observed in infected individuals (Fig. 12) and the reduced competitive ability when growing in low nutrient conditions (Fig. 11; a feature typical of the study sites) suggests that a similar sensitivity to harsh conditions may well occur in *S. amphilophis* infected *B. macra.*

An alternative explanation of the regional distribution of disease as an ephemeral event, resulting from the migration of disease from north to south, seems unlikely given the stability of the position of the boundary between heavy and low/no infection. In addition, the only two southern populations assessed in both 1981 and 1993 (Williamsdale and Bunyan) were disease-free at both times.

Local variation in disease occurrence

The first part of this study strongly suggests that the physical environment, particularly temperature, restrains pathogen development on a regional scale. At the local population level, however, biotic factors associated with competition play a significant role in the occurrence and spatial distribution of infected individuals.

In all five populations studied in detail, positive relationships existed between levels of infection and the density and spatial position of plants within the population (edge versus core area) (Fig. 9; Table 1). Host density has been shown to be significantly correlated with disease severity in a wide range of associations (Burdon and Chilvers 1982) and where the relationship is positive, pathogens could be important in the regulation of host population size (Antonovics and Levin 1980). However, as Burdon and Chilvers (1982) pointed out, density-dependent relationships may arise in a variety of ways. Without precise knowledge of the mechanism of infection of *B. macra* by *S. amphilophis* explanation of the current positive density-dependent association will remain speculative.

In smut-host plant interactions in which adult plants may become infected [for example, *Silene* spp. with *Microbotryum violaceum* (*Ustilago violacea*)], the development of density or at least frequency-dependent relationships are easily explained (Antonovics and Alexander 1992; Carlsson and Elmqvist 1992). Grass-smut interactions, on the other hand, differ from these in that infection of previously healthy adult plants does not occur. As a consequence, there are only two obvious explanations for the observed relationship: either (i) differential survival of infected over healthy individuals; or (ii) continuing recruitment occurring within populations in which later occurring individuals would have sequentially higher probabilities of being infected simply by virtue of the presence of diseased plants in the extant stand. These possibilities are not mutually exclusive and may both contribute to the observed patterns of distribution of smut-infected plants within individual populations.

In four of the five sites there was marked spatial patterning in the distribution of infected plants (Fig. 8). In these populations, disease incidence was greater in the edge than the core area. Edge areas of the populations were subject to more disturbance (due to their proximity to paths or roadside verges) than the core areas. This provides continuing opportunity for the germination and establishment of new *B. macra* plants which, regardless of the mode of infection by *S. amphilophis*, will have a higher probability of infection due to the close proximity of pre-existing infected plants acting as sources of inoculum.

In contrast, in the less disturbed core areas of populations growing on low nutrient sites (typical of the entire area), healthy plants are likely to have a competitive advantage over infected ones and as shown in the glasshouse experiments will tend to dominate (Fig. 11). The only site which failed to show a difference in percent infection between the plants growing in the edge and core areas was a long, narrow population (Uriarra Road) that was effectively all edge.

The long-term evolutionary interaction between *S. amphilophis* and *B. macra*

Systemic floral-smut pathogens have profound effects on their hosts frequently reducing seed production to zero. In this respect the *Sporisorium amphilophis* infection of *Bothriochloa macra* is no different to many other smut infections of both monocotyledonous and dicotyledonous plants (Falloon *et al.* 1988; Alexander 1989). In addition, however, many of these pathogens also induce changes in the morphology of infected hosts that assist in the transmission of disease to healthy individuals. Thus *Silene alba* systematically infected by *Microbotryum violaceum* produces more flowers than do healthy individuals. In that association a simple explanation for these morphological changes can be found in the increased probability of disease transmission that results from increased pollinator (=vector) visitation (Alexander and Maltby 1990). In the interaction between *S. amphilophis* and *Bothriochloa macra*, infection also leads to a significant increase in the number of inflorescences. It is reasonable to assume that this also increases the chances of successful transmission (through increased spore production) and energetically is at least partly compensated for by their decreased height.

The balance struck between the interplay of biotic and abiotic factors is vital in determining the intensity of host-pathogen interactions and hence their long-term consequences. In the colder parts of the survey area, *S. amphilophis* is largely absent in populations of *B. macra*. Such environmentally-based refugia have been reported previously in other host-pathogen associations (for example, *Thlaspi alpestre*; Rochow 1970). Indeed, such systematic patterns in environmental conditions may lead to marked differences in the genetic structure of host populations occurring in different areas. This has been found on a number of occasions for foliar diseases where the incidence of resistance to *Puccinia coronata* in *Avena* spp. and *Rhynchosporium secalis* in *Hordeum spontaneum* is greater in mesic areas of Israel that favour pathogen development than in more arid ones (Dinoor 1970; Abbott *et al.* 1992).

Until the mode of infection of *B. macra* by *S. amphilophis* is determined, detailed studies of the genetics of this interaction will not be possible. However, my failure to find many populations in which infection levels exceeded 50% may reflect the presence of resistant hosts. Even if this is not the case (and these individuals simply represent instances of disease escape), the density-dependent relationship between percent infection and host density indicates that the pathogen may be an important component of plant population regulation at some sites.

CHAPTER 5

Mechanism of infection of the native grass *Bothriochloa macra* (Steud) S.T. Blake by the floral-smut fungus *Sporisorium amphilophis* (Syd.) Langdon and Fullerton

INTRODUCTION

The process of infection of a host plant by a pathogen is the most vital in the plethora of interactions that are an integral part of all host-pathogen associations. For the many pathogens whose symptoms become apparent within a few days or weeks of infecting their hosts (eg. many foliar diseases, damping-off fungi), the site and mode of infection, and the environmental requirements for spore germination and penetration are usually relatively simple to determine. However, disease symptoms induced by many other pathogens may not be apparent for months or years. Indeed, infection may occur at one stage of the host life-cycle but not become apparent until another stage or even the following generation. Systemic floral smut fungi are good examples of this phenomenon. While the mechanisms of infection has been worked out in many cases (Nielsen 1988; Singh and Krishna 1982; Thakur 1989), in others including some agriculturally important pathogens, much still remains to be uncovered. Floral smuts are particularly devastating pathogens since infection usually results in host sterility with the floral organs being replaced by a mass of fungal teliospores. Within the order Ustilaginales (smuts) there are at least four modes of entry of these pathogens into their host plants (Punithalingam 1971):

1. Infection of germinating seed.
2. Infection of young plants (seedlings) or young growth.
3. Infection of current adult generation through the flower.
4. Infection of next generation through the flower-ovule-seed.

These mechanisms have been shown to occur in a variety of plants - some of which are given in Table 1. However, they are not entirely mutually exclusive with for example, modes 1 and 2 commonly occurring in the same pathogen-host

combination. Equally though, infection of current adult generation plants (mode 3) has never been reported in monocotyledonous plants although it is known in dicotyledonous plants, for example *Microbotryum violaceum* infecting *Silene alba* in North America (Alexander 1990b) and *Silene dioica* in Northern Sweden (Carlsson and Elmqvist 1992).

In monocotyledonous plants, infection modes [1] and [4] are well established although [1] seems to be far the commonest mechanism. In mechanism 1 teliospores that overwinter either on contaminated seed or in the soil, germinate and produce basidiospores during the spring which may then be dispersed by wind or water to young emerging tissues of host plants. In some smut species, budding of basidiospores takes place and two basidiospores of opposite strains mate. In other species, basidiospores germinate on the host surface and produce a fine hypha which can enter epidermal cells by direct penetration. There they may contact and fuse with a haploid hypha derived from a basidiospore of a compatible mating type. The resulting dikaryotic hypha enlarges in diameter and grows mostly intercellularly, within the plant. The pathogen frequently invades the meristematic region of the plant occupying new plant tissues as they develop. Sori of teliospores form in place of the floral parts (Alexopoulos and Mims 1979; Thomas 1988).

The infection process involving smuts that follow infection mode 4 begins when teliospores released by previously infected plants land on the stigmatic surfaces of flowers of healthy plants. There the teliospores germinate to form a basidium on which the haploid hyphae are produced. Fusion of sexually compatible haploid hyphae, results in a dikaryotic mycelium. If these developments occur on a healthy flower the mycelium penetrates through the stigma or young ovary walls and becomes established in the pericarp and tissues of the embryo before the seeds become mature. The mycelium then becomes inactive and remains dormant, primarily in the scutellum, until the infected seed germinates (Agrios 1978).

To date, it is not known by what route the systemic floral-smut fungus *Sporisorium amphilophis* gains entry and infects its host, *Bothriochloa macra.* Here I investigate possible mechanisms of infection through a series of glasshouse experiments involving inoculation of five stages of the life cycle of *B. macra,* from germination to anthesis with teliospores and sporidia of *S. amphilophis* (Table 2).

Table 1. Representative examples of the modes of infection found in a variety of host-smut pathogen associations.

Infection pathways	Host-pathogen association	References
Germinating seed	*Bromus willdenowii - Ustilago bullata*	Falloon and Hume (1988)
	Wheat - *Tilletia caries*	Luttrell (1981)
	Barley - *Ustilago hordei*	Thomas (1988)
	Onion - *Urocystis cepulae*	Croxall and Hickman (1953)
	Sorghum halepense - Sphacelotheca holci	Massion and Lindow (1986)
Young plants (seedlings) or young growth	*Bromus willdenowii - Ustilago bullata*	Falloon and Hume (1988)
	Silene alba - Ustilago violacea (Microbotryum violaceum)	Thrall *et al.* (1993)
	Silene dioica - Ustilago violacea	Carlsson and Elmqvist (1992)
	Sorghum halepense - Sphacelotheca holci	Massion and Lindow (1986)
	Sugar cane - *Ustilago scitaminea*	Hoy *et al.* (1991)
Current adult generation through flower	*Silene alba - Ustilago violacea (Microbotryum violaceum)*	Thrall *et al.* (1993) Spencer and White (1951)
	Silene dioica - Ustilago violacea (Microbotryum violaceum)	Carlsson and Elmqvist (1992)
	Lychnis viscaria - Ustilago violacea (Microbotryum violaceum)	Ingvarsson and Lundberg (1993)
Next generation through the flower-ovule	Barley - *Ustilago nuda*	Doling (1964)
	Wheat - *Ustilago tritici*	Nielsen (1988)
	Pearl millet - *Tolyposporium penicillariae*	Thakur (1989)

HOST-PATHOGEN SYSTEM

Bothriochloa macra (Steud) S.T. Blake is a native perennial grass that is found in grasslands and woodlands, usually on loams and clays, in eastern Australia. This grass often survives in heavily-grazed native pasture and is frequently found colonising disturbed areas. It is considered a valuable grass for soil conservation purposes (Lamp *et al.* 1990). The main flowering time for *B. macra* is early summer (November-January), and the seed is shed as it become ripe until late April. Germination typically occurs in late spring (Hagon 1976).

Bothriochloa macra is often infected by the flower-smut fungus *Sporisorium amphilophis* (Syd.) Langdon and Fullerton. As in other systemic floral smuts, this pathogen affects the fitness of infected plants, since it replace the host's seed production with a mass of spores. The disease also reduces the size of the infected plants and leads to an increase in the number of inflorescences per plant (cf. Chapter 4).

METHODOLOGY

Possible mechanisms of infection of *B. macra* by *S. amphilophis* were examined at five stages in the host life-cycle (Table 2). For all the experiments, teliospores used as inoculum were taken directly from several infected inflorescences and mixed to ensure the presence of compatible mating strains. Seed were obtained from healthy plants growing in natural populations near Canberra.

Experiments

1. Inoculation of seeds: 0.5 g of *B. macra* seed were vacuum-inoculated for 15 minutes with a suspension of *S. amphilophis* teliospores at two concentrations (0.002 and 0.004 g of spores/ L distilled water). After inoculation seeds were kept in the spore suspension for 24 hours. The following day 5 seeds from each treatment and controls were sown in separate pots filled with potting compost (5 seeds/pot), and maintained in a naturally-lit glasshouse (18-24 °C) until flowering occurred. Each treatment was replicated 5 times. Control plants were treated in the same way as inoculated plants except that sterile distilled water was used instead of the teliospore suspension.

Table 2. Different stages in the life-cycle of *Bothriochloa macra* at which infection by *Sporisorium amphilophis* was attempted.

Stage of host life-cycle	Experiment Number
Germinating seed	1, 6 and 7
Seedling	2 and 3
Vegetative plant	3
Developing inflorescence (boot stage)	4 and 8
Anthesis (via stigma)	5

2. Inoculation through the seedling coleoptile and radicle:

(a) *Coleoptile*. Seedlings were inoculated during the first day of coleoptile emergence in two different ways:

(i) infection of wounds: the coleoptile of 10 seedlings was punctured once with a dissecting needle which had been coated with dry teliospores; and
(ii) direct contact: dry teliospores were spread over the coleoptile of 10 seedlings using a small brush.

(b) *Radicle*. For the inoculation of the radicle the same methodology was followed. In both cases inoculated seedlings were individually planted in 15 cm pots filled with potting compost, covered with plastic bags for 24 hours, to

increase humidity, and maintained in a naturally-lit glasshouse (18-24 °C) until flowering occurred.

Control treatments for both coleoptile or radicle inoculation treatments consisted of puncturing seedlings with a sterile needle or brushing with a clean brush.

3. Inoculation of young and growing portions of shoots: Seedlings were inoculated in two ways: (i) physical damage: a dissecting needle was dipped into a spore suspension (0.004 g of spores/litre distilled water) and used to puncture the base of the stems of ten seedlings, (ii) direct penetration: a suspension of spores was sprayed over the shoots of ten seedlings until run off.

To assess the 'infectability' of vegetative plants, 30 plants in the tiller development phase of growth were inoculated by 4 different means (ten plants per treatment): (i) physical damage (same methodology as used above using a spore suspension); (ii) direct contact: a cotton pad moistened with the spore suspension was placed in direct contact with young tillers for 24 hours; (iii) injection of teliospore suspension into the young tillers; and (iv) spraying of the teliospore suspension over shoots. In all cases a suspension of 0.004 g of spores /litre was used. Following treatment, each plant was covered with a plastic bag for 24 hours, to increase humidity and to avoid the spores being washed away by watering. Subsequently all plants were maintained in a glasshouse (18-24 °C) until flowering.

Appropriate control treatments were carried out utilising the same methodology but without the presence of teliospores.

4. Inoculation of young inflorescences: The 'boot' stage of 15 plants were inoculated with a 0.004 g/l spore suspension by three different means (5 plants per treatment): (i) physical damage: puncturing the inflorescence three times in different areas with a dissecting needle into the teliospore suspension; (ii) direct contact: placing a cotton pad wetted with the spore suspension around the inflorescence for 24 hours; and iii) injection of the spore suspension into the young inflorescence.

Control treatments were carried out using sterile distilled water instead of the spore suspension.

5. Inoculation of the stigma: Dry teliospores of *S. amphilophis* were dusted onto stigmas of ten flowers on five plants during the first day of flower opening. Each plant was covered with a plastic bag for 24 hours, to increase humidity and to avoid the spores being washed away by watering. All the plants were maintained in the glasshouse until mature seeds were obtained. The seed from these plants were germinated in Petri dishes and then transplanted to pots (5 seeds per pot) filled with potting compost and kept in a glasshouse (18-24 °C) until flowering occurred. Control treatments consisted of brushing stigmas with a clean brush.

Infection of host tissue does not occur directly from germinating teliospores. Rather it requires the fusion of sexually compatible haploid hyphae produced through the germination of different basidiospores derived from teliospores. In order to ensure the presence of such infective structures an additional treatment to experiments 1 to 5 included the use of conidial suspensions obtained from purified cultures of *S. amphilophis* as inoculum. These cultures were derived by isolating individual basidiospores of *S. amphilophis* and culturing these on Potato-Dextrose-Agar (PDA) media. The sporidial suspension used for inoculation was obtained by 'washing' and mixing sporidia from 5 different cultures with sterile distilled water, in order to assure encounter among compatible sporidia. For all the treatments control plants were inoculated in the same path used for each test but using sterile distilled water instead of the fungal suspension.

All the experiments described above (1-5) failed to produce infected plants. Observations on the distribution of this floral-smut in the field suggested a relationship between higher incidence of this disease and higher temperatures (Chapter 4), so a second set of experiments (6-9), aimed to investigate the possible occurrence of a temperature window for infection. In these experiments inoculated and uninoculated seed and plants were kept in naturally-lit phytotron chambers with the following range of day/night temperatures: 17/7, 20/10, 23/13, 26/16, 29/19 and 32/21°C. In all experiments *B. macra* seeds were sown, 5 to a pot in 15 cm pots filled with potting compost.

6. Inoculation of seed: 0.5 g of *B. macra* seed were vacuum -inoculated with a 0.004 g/ L teliospore suspension and left in the suspension for 24 hours. A control, uninoculated treatment was provided by soaking 0.5 g of seed in sterile distilled water for 24 hours. After planting three replicates of each treatment were kept at each of 6 temperatures regimes in the phytotron for 12 days. The

time to emergence of each seedling was recorded. Once all the seedlings emerged, the pots were transferred to a naturally-lit glasshouse (18-24 °C), until flowering occurred.

7. Inoculation of inflorescences: *B. macra* seedlings obtained from seed collected from healthy plants in the field were planted 5 to a pot in 15 cm pots filled with potting compost. All plants were kept in a naturally lit glasshouse until 'boot' stage. At this stage plants were inoculated by injecting a spore suspension into the 'boot'. Plants were then transferred to each of the 6 phytotron temperature regimes and kept there until seed production occurred. Each treatment was replicated 5 times. Seed from each treatment was collected when mature, sown in plastic flats filled with potting compost and these plants kept in a naturally-lit glasshouse until flowering.

8. Effect of temperature on *S. amphilophis* in vitro: The objective of this experiment was to determine the effect of a range of temperatures on the growth of *S. amphilophis*. in culture. Teliospore samples were collected from naturally infected inflorescences of *B. macra* in the field and spread sparsely on Petri dishes with PDA medium and 0.03g of streptomycin per litre of medium, to avoid plates being overrun by bacteria. Five sets of 10 Petri dishes were obtained in this way and assigned to an incubation temperature of 10, 15, 20, 25 or 30°C. The Petri dishes were analysed every 24 hours for a period of 10 days and the development and growth of *S. amphilophis* colonies recorded. Dry spores collected from the field and preserved with silica gel in sealed jars, after two to three weeks, rarely germinated.

RESULTS

None of the experiments succeeded in producing infected plants, instead inoculated and uninoculated seedlings grew to maturity and produced healthy flowers.

Experiment 6. This experiment did not show any significant difference in the emergence of inoculated and inoculated seedlings (logistic regression; $P > 0.05$). Both sort of seedlings emerged 11 to 14 days after sowing under all temperature regimes, but there was no evidence of a consistent trend or particular optimum temperature for earlier seed germination. In addition, temperature had a marginal significant effect on the proportion of emergence of all kind of seedlings ($P >$

0.02). The highest emergence of seedlings was at 32/21°C and the lowest at 17/7°C and 20/10°C (Fig. 1).

Experiment 9

S. amphilophis teliospores, produced small yeast-like colonies after 24 hours at 20, 25 and 30°C. There was no observed development of *S. amphilophis* colonies in any of the Petri dishes kept at 10 and 15°C.

DISCUSSION

This study suggests that *S. amphilophis* is likely to have very specific requirements for infection of *B. macra.* None of the experiment approaches used were successful in generating infected plants even though several of these techniques are always associated with a high degree of success when applied to appropriate pathogen-host combinations. For example, barley and wheat inoculated at anthesis with *Ustilago nuda* and *Ustilago tritici* respectively (Nielsen 1988), inoculation of *Silene alba* with *Ustilago violacea (Microbotryum violaceum)* at seedling stage (Alexander and Maltby 1990), inoculation of *Bromus catharticus* (Falloon 1976; Chapter 2) and *Agropyron scabrum* with *Ustilago bullata* at seed germination stage (Kirby 1987), and inoculation of shoots, seedlings and coleoptiles of *Bromus catharticus* with *Ustilago bullata* (Falloon 1979b).

Successful infection of hosts by pathogens depends on many biotic and abiotic factors including favourable temperature and moisture conditions, susceptibility of the host, aggressiveness of the pathogen, effects of other microorganisms present, stimulatory or inhibitory substances secreted by the host plant, or quality of the host tissue (Agrios 1978, Tarr 1972). In particular many smut fungi show marked temperature sensitivity. Indeed, for many agricultural field crops the importance of sowing dates to lessen risk of smut disease has been long recognised. Thus the incidence of the smut fungus *Tilletia caries* can be 40 to 60% higher when winter wheat is sown from mid September to mid October (Palti 1981) than earlier or later. Likewise for *Ustilago hordei* growing in the United States of America it has been reported that soil conditions become more favourable for infection of winter barleys from early September to late October, beyond this period cold temperatures become the limiting factor in infection (Tapke 1948). In the current study, experiment 9 showed that *S. amphilophis* growth *in vitro* is adversely affected by low temperatures. This suggests the requirement of

optimum temperatures (20-30 °C) for successful infection to occur. However, the set of experiments involving growing inoculated plants in a series of temperature regimes (experiments 6 and 7) found no evidence of infection, and all other experiments were conducted at about 20 °C so temperature should not have been limiting.

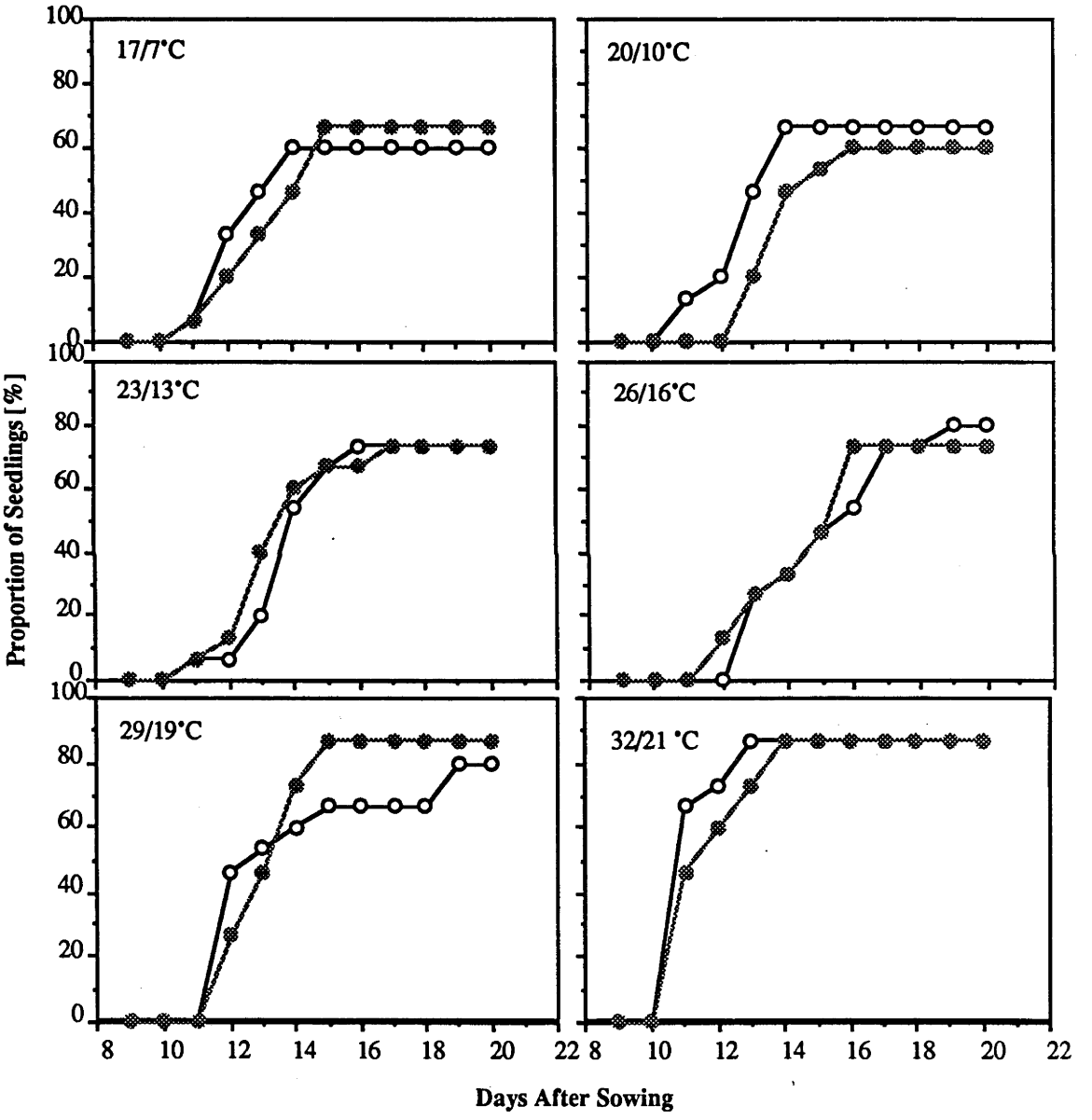


Fig. 1. Percentage germination of *B. macra* inoculated and uninoculated with the smut fungus *S. amphilophis* under six temperature regimes.
—○— uninoculated, —●— inoculated.

Inoculation experiments involving coating seeds with teliospores have shown this to be a procedure that ensures near 100% infection in many smut species (eg. *Bromus catharticus* by *Ustilago bullata* and *Cynodon dactylon* by *Ustilago cynodontis*). The failure of this approach to infect *B. macra* in this study can not be attributed to low teliospore viability since they were freshly collected from infected inflorescences of several plants from at least 5 different populations, and when grown on PDA medium, readily produced smut colonies. Similarly it seems highly unlikely that the *B. macra* seed was genetically resistant to infection as it was collected from several different populations where disease incidence ranged from 20 to 40%.

Inoculation through physical damage of stems have been proved to be a very effective technique for some systemic smuts (eg. *Sphacelotheca holci* infecting *Sorghum halepense*; Massion and Lindow 1986). However, there is some evidence that environmental factors may have a strong influence when this kind of inoculation is attempted. For example, it has been reported that in some cases free moisture is required for infection of *Sorghum halepense* by *Sphacelotheca holci* (Massion and Lindow 1986). In my study (experiment 3) it is possible that moisture or temperature were not appropriate for infection to occur though conditions of moisture and temperature seemed appropriate.There is a possibility that the use of a dissecting needle dipped into a spore suspension did not bring adequate inoculum levels into the plants. However, the direct injection of teliospore or sporidial suspension into the tillers assured high levels of inoculum as well as free moisture. Again the procedure was unsuccessful.

Previous evidence has shown that experiments involving floral inoculation of grasses have to be carried out by depositing spores either on the ovary or stigma during anthesis (Punithalingam 1971). Artificial inoculation involves several techniques such as spraying or injecting the flowers with spore suspensions or injecting dry teliospores into the flowers one or two days after anthesis (eg. *Ustilago nuda* in barley; Punithalingam 1971). For some other embryo-infecting smut species such as *Ustilago tritici* (loose smut of wheat), successful inoculation requires a mixture of compatible monokaryotic haploid basidiospores be brought into the flowers at anthesis, so they can fuse to form dikaryons soon after injection into the flowers (Nielsen 1988). My study did not involved the direct inoculation of the ovary, however injection of the boot stage with teliospores and sporidia

suspensions has been proved to be a successful inoculation method for some smut species. For example *Pennisetum glaucum* can be inoculated with the smut *Tolyposporium penicillarie* by injecting a sporidia suspension into the boot leaf sheet and covering the inflorescences with parchment bags (Thakur 1989). This approach did not work with *S. amphilophis*. Similarly direct inoculation of the stigmatic surface with teliospores also failed to induce infection of developing seed.

S. amphilophis infection levels in natural populations of *B. macra* have been observed to be variable, but the incidence of disease can reach more than 50% (Chapter 4), in areas where few days with frost occurring during winter period. In this study I tried several inoculation techniques that may possibly occur in natural conditions None of these were successful. The incorrect abiotic conditions may been applied to test seed infection but they seemed appropriate. Given the difficulty of infection it is suspected that infection of *B. macra* by *S. amphilophis* probably occurs through floral structures, Confirmation or rejection of this belief will require further studies involving careful manipulation of temperature, moisture and inoculation of flowers at different stages of development. The role of phalacrid beetles (Chapter 6) also deserves attention as a vector which might visit flowers at an early stage of floral development.

CHAPTER 6

Phalacrid beetles and their interaction with the floral systemic smut *Sporisorium amphilophis* (Syd.) Langdon and Fullerton

INTRODUCTION

The range of interactions between insects and plant pathogens is almost as diverse as the range of plant pathogens. Crudely, these interactions can be arrayed along a continuum ranging from simple situations where pathogens utilise insects as means of enhancing or ensuring the transmission of spores from one host plant to another, to the other extreme where insects play no role in transmission but utilise the pathogen as a food source. Between these two extremes are a range of complex, potentially symbiotic interactions in which both pathogen and insect gain from an association. Perhaps the best known of these is the pathogen-insect interaction that lies at the heart of the spread of *Ophiostoma ulmi* (Dutch elm disease). In this instance, dispersal of this pathogen relies on its interaction with bark beetles (*Scolytus multistriatus* and *Hylurgopinus rufipes*). When these beetles lay their eggs on suitable hosts they also inoculate the host with the fungus. The insect larvae subsequently feed on the fungus within the infected tree (Agrios 1978). Other plant pathogens such as the rust *Puccinia monoica* infecting *Arabis* spp. can transform host morphology into flower-like structures on which the pathogen produces nectar, scent and spermatia to attract pollinators which spread the disease (Roy 1993). Finally, at the other extreme of insect-pathogen interactions are a number of examples where insects simply 'graze' fungal lesions but play no role in transmission. An example of this behaviour occurs with the mycophagous beetle *Aclyomus* sp. feeding on the ergot fungus *Claviceps purpurea* infecting *Festuca arundinacea* (Lemon 1992).

Smut fungi similarly show a range of interactions with insects that cover most of this continuum. For many smut fungi, particularly those involving systemic floral infection of non-wind pollinated plants, insects are a vital component of the transmission cycle. Examples of such interactions are seen occurring between caryophyllaceous plants and the floral smut *Ustilago violacea* (Jennersten 1983;

Alexander 1990a; Carlsson and Elmqvist 1992) which replaces the ovary and anthers of the host with a fungal stroma so that insects visiting smut infected flowers in search of nectar or pollen are dusted with spores which they may subsequently carry to other plants.

Amongst smut diseases of monocotyledonous plants such as sedges and grasses, where wind-pollination predominates, interactions involving insects are again quite common. One of the most widespread interactions is that involving beetles of the genus *Phalacrus* (Coleoptera, Phalacridae). Many of these beetles are mycophagous but show a range of interactions with fungi ranging from situations where they have no apparent role in the infection process (eg. *P. politus* in galls of corn smut; Steiner 1984) to situations where they are important enhancers of infection. A good example of the latter situation involves the non-systemic floral smut *Anthracoidea fischeri* infecting *Carex* spp. in Sweden (Ericson *et al.* 1993). In that interaction, infection of *Carex* florets does occur in the absence of the beetle but is always substantially lower than that occurring when the beetle is present.

During preliminary survey work on the *Bothriochloa macra - Sporisorium amphilophis* interaction occurring in south-east Australia I frequently encountered *Phalacrus* insects. This chapter reports a series of observations concerning the beetles. These were carried out to determine the possible role of the phalacrid beetle *Phalacrus* sp. in the dynamics of smut infection on *B. macra*. Specifically, the study aimed to: (i) determine the abundance of *Phalacrus* sp. adults and larvae, in populations of the grass *B. macra* infected by the flower-smut fungus *Sporisorium amphilophis*, and (ii) determine their possible role in the transport of smut spores.

STUDY SPECIES

Bothriochloa macra -Sporisorium amphilophis

Bothriochloa macra (Steud.) S.T. Blake is a native perennial grass naturally found in a range of native grasslands. It is favoured by over-grazing and often colonises disturbed areas in eastern Australia. Established plants increase in size by tillering (Lamp *et al.* 1990; Maze *et al.* 1993). The main flowering time is early summer, although inflorescences can be found from November to April, and most seed is shed in April. Germination mostly occurs in late spring (October-November) when soil temperatures are high enough to enable rapid germination and seedling establishment (Hagon, 1976).

Sporisorium amphilophis (Syd.) Langdon & Fullerton is a perennial, systemic floral-smut fungus that is specific to *B. macra*. Infected plants thereby produce a crop of spores each year. The mechanism of infection of *B. macra* by *S. amphilophis* is unknown (Chapter 5). This pathogen reduces plant fitness by replacing all inflorescences with a mass of spores. In the field infected plants are smaller and produce more inflorescences than do healthy individuals (Chapter 4). Smut-infected flower heads are found from November to April, at the time of normal flower production.

Phalacrus sp.

Very little is known about the *Phalacrus* species (Phalacridae, Coleoptera) associated with smut infected plants in general. However even less is known about the species associated with *B. macra* smutted inflorescences in Australia. This as yet unnamed species of *Phalacrus*, is a small shiny black beetle frequently found in association with smut-infected inflorescences of *B. macra*. The adults are 2 to 3 mm long, with a body form that has a nearly round to oval dorsal outline but is flat ventrally (Fig. 1). As seen in Fig. 2, the legs are covered with seta which may trap spores.

Nothing is known about the life-cycle of this *Phalacrus* sp. on *B. macra*. However, for other species of the genus it is known that the entire life cycle of the beetle and its immature stages (egg, larvae and pupa) may be passed either within the smutted head or on the leaves of the host plant (eg. *Phalacrus immarginatus* feeding on sugarcane smut; Agarwal 1956). In contrast, adults of species such as *P. substriatus* (feeding on *Carex* spp smut; Ericson *et al.* 1993) and *P. politus* (feeding on smut of corn; Steiner 1984) appear at the onset of flowering and feed on smut sori in the inflorescences. Egg deposition and larval behaviour is similarly variable.

In the case of *P. politus* (feeding on corn smut), eggs are deposited in crevices between galls, between galls and husk, or in splits in the outer tissue of the galls. In the case of *P. substriatus* eggs are laid on the outer surface of sori or in a hole bitten in the perigynium. In *P. substriatus* larvae hatch after about one week and feed internally in the smut sorus. In *P. immarginatus* the larval stage last from 20-26 days (Agarwal 1956), while for *P. politus* it last from 10 to 14 days (Steiner 1984). Pupation usually occurs in the sorus (eg. *P. substriatus*) but in some species such as *P. immarginatus*, it occurs in the transverse mark at the base of the leaf near its junction with the stem inside (Agarwal 1956).

Fig. 1. Scanning electron micrographs of *Phalacrus* sp. (A) Dorsal view, (B) ventral view, (C) lateral view.

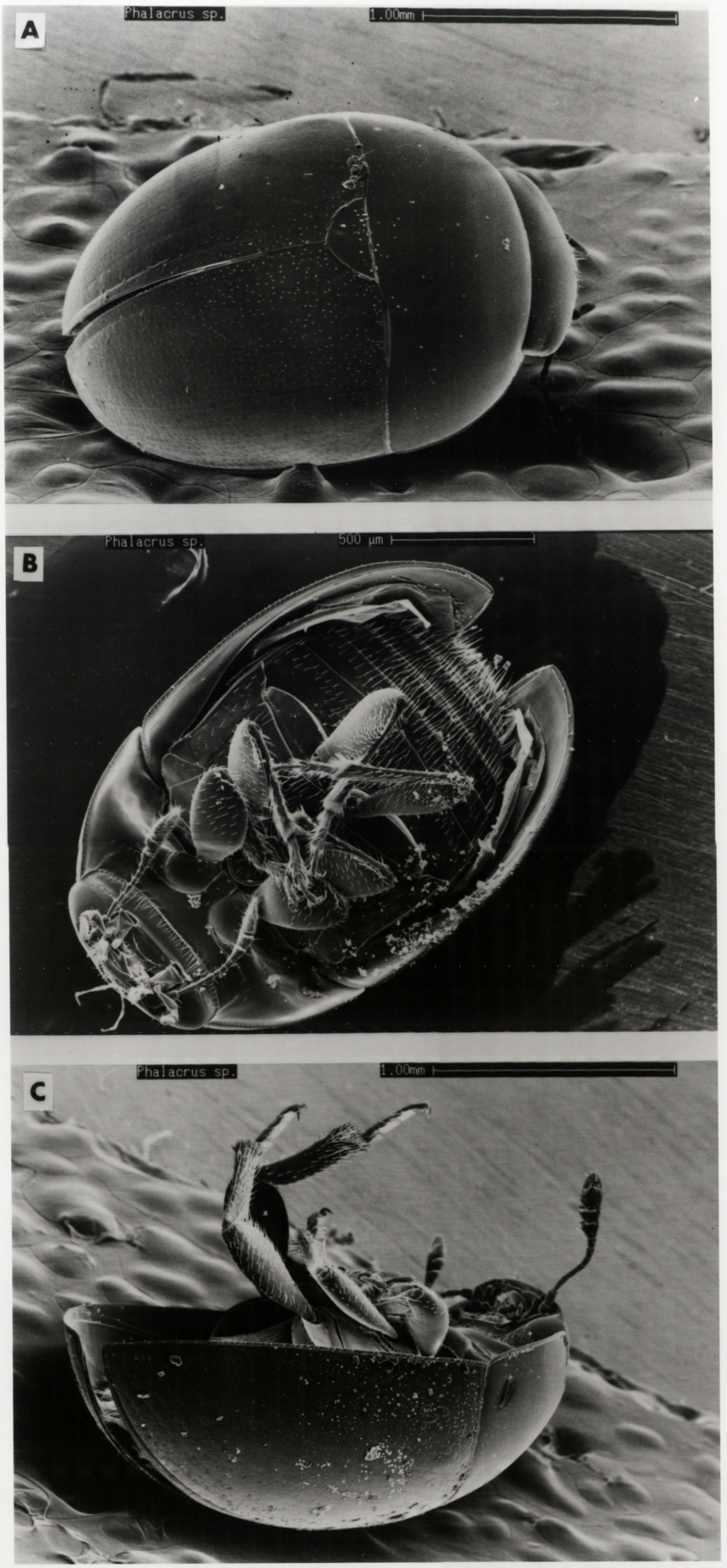
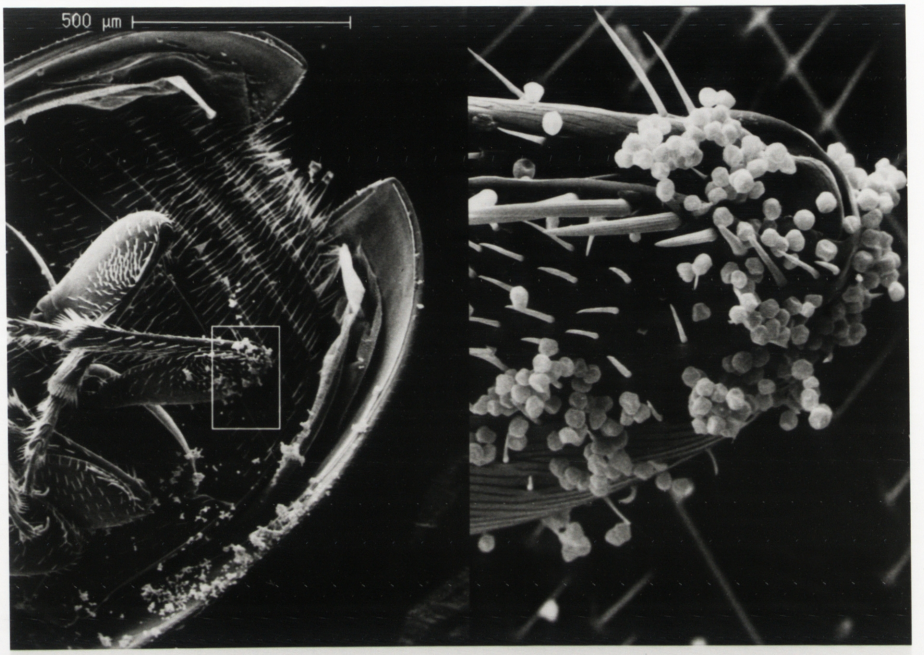

A
Phalacrus sp.
1.00mm
B
Phalacrus sp.
500 µm
C
Phalacrus sp.
1.00mm

Fig. 2. Scanning electron micrographs of *Phalacrus* sp. abdominal area, showing legs covered with seta and teliospores of *S. amphilophis*.


500 µm


Phalacrus sp.
10.0µm

The emergence of adults can occur after 4-6 days in species such as *P. politus* or after three weeks in *P. substriatus* (Ericson *et al.* 1993). Many species appear to overwinter in the adult stage (eg. *P. substriatus* or *P. politus*) and feed on overwintering teliospores on the ground (Ericson *et al.* 1993).

STUDY SITES

Field work was carried out during May 1993, and between November 1994 and March 1995, in six and five *B. macra* populations respectively. These populations were located in the Australian Capital Territory and adjacent region of the New South Wales Southern Tablelands (Fig. 3) and were selected as a result of previous observations which suggested that *Phalacrus* was present in all populations wherever smut infection occurred. The size of individual *B. macra* populations examined in the surveys ranged from several hundreds to many thousands of plants. The incidence of smut disease in these populations was determined by selecting 100 *B. macra* plants at random and scoring their disease status (healthy or infected; cf. Chapter 4).

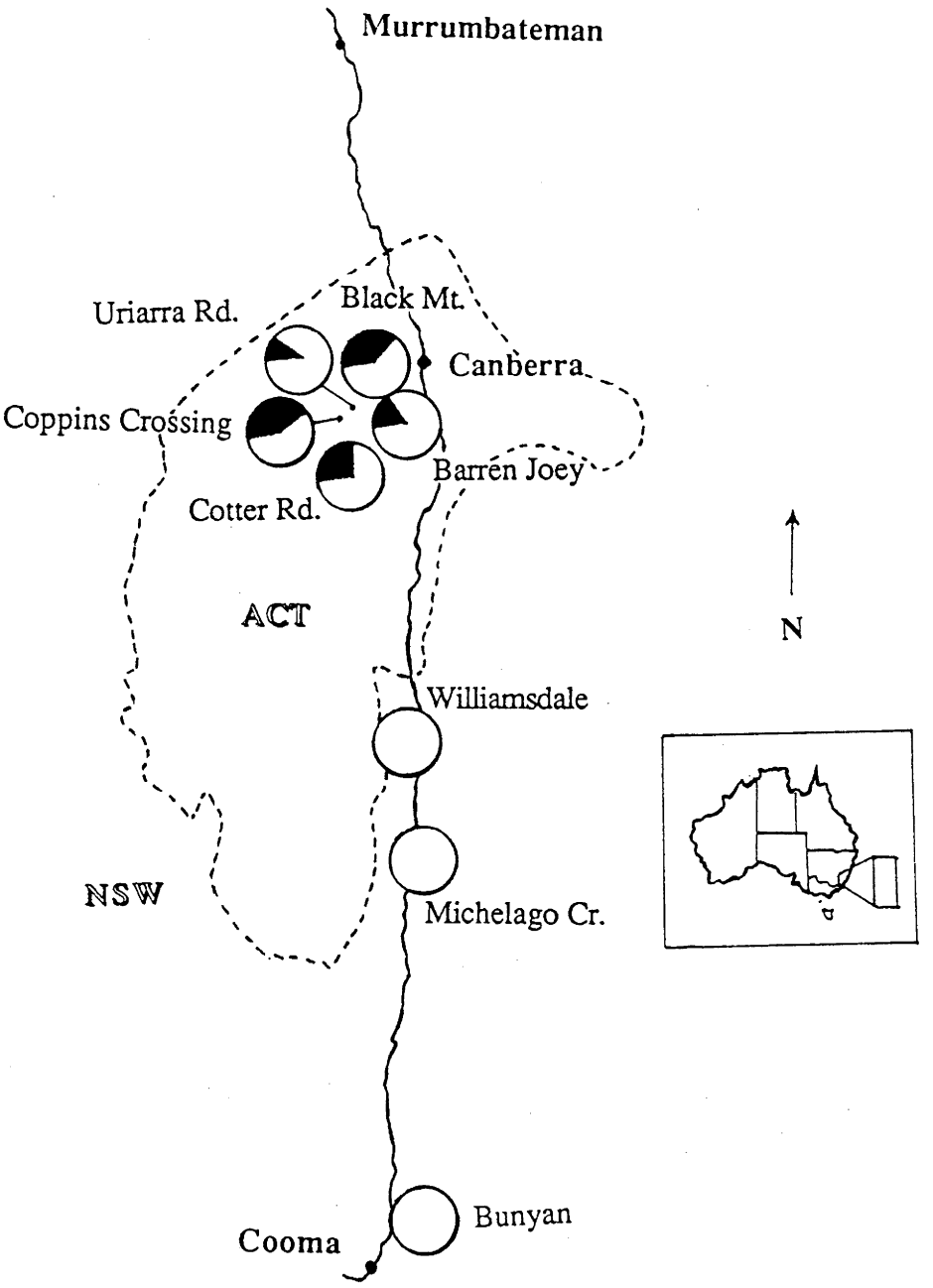
METHODS

Field observations

(a) *Incidence of adult* Phalacrus *in the field*

To determine the incidence of adult beetles in the field, I carried out two surveys. The first (Survey 1), conducted in May 1993, examined the abundance of beetles and its relation with smut disease incidence in six *B. macra* populations (Fig. 3). These populations, located in the ACT, were characterised by higher levels of smut infection (19-45%), while the other half, located in adjacent region of the Southern Tablelands of New South Wales were distinguished by populations where smut disease was absent. The second survey (Survey 2) spanned the entire *B. macra* flowering season of 1994-1995. The survey began in November when *B. macra* plants started to flower and finished in March 1995 when seed were maturing. This survey looked at temporal variation in the abundance of beetles in five *B. macra* populations located in the ACT area. For both surveys, 5 sets of 20 sweeps each were carried out at inflorescence height, with a 30 cm diameter insect net. Each population was sampled by walking in a zigzag fashion across the stand. All parts of the *B. macra* populations

Fig. 3. Geographic distribution of 8 populations of *B. macra*, with the percentage of smut-infection for each population shown by black portion of pie diagram.


Murrumbateman
Uriarra Rd.
Black Mt.
Canberra
Coppins Crossing
Barren Joey
Cotter Rd.
ACT
N
Williamsdale
NSW
Michelago Cr.
Bunyan
Cooma

were covered. Each sweep included 5-15 inflorescences. The net was emptied after each set and the number of *Phalacrus* individuals recorded.

(b) *Larval incidence in the field*

To determine larval incidence in the field, in May 1993 100 infected and 100 healthy *B. macra* inflorescences were randomly collected after anthesis from the six *B. macra* populations where adult beetles were observed. Inflorescences were dissected, and the number and position of larvae per inflorescence recorded.

(c) *Does* Phalacrus *feed on the smut?*

To determine whether adult beetles and larvae feed on smut spores, 10 adults and 10 larvae were collected from infected inflorescences of *B. macra* and their gut examined for smut spores. The guts were removed mounted on microscope slides and stained with alcoholic lactophenol cotton blue.

Laboratory observations

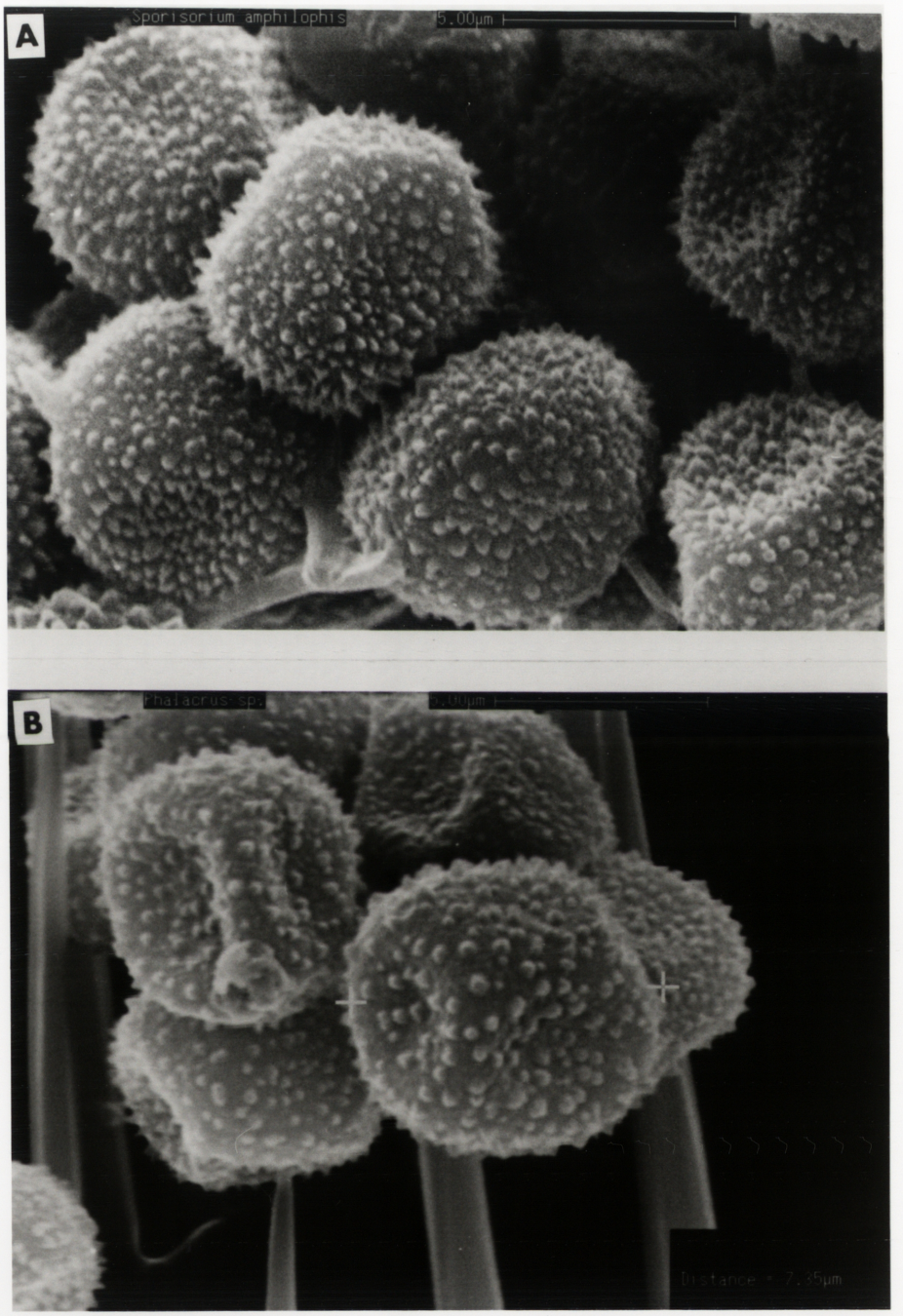
Scanning Electron Microscope (SEM) examination for presence of spores

The bodies of 5 *Phalacrus* sp. beetles collected from the field were externally searched for *S. amphilophis* teliospores using a scanning electron microscope (SEM). The beetles were mounted on pin stubs with silver paint, sputer-coated with gold, and viewed and photographed with a Cambridge S360 scanning electron microscope. The identification of *S. amphilophis* teliospores was confirmed by comparing teliospores found on beetles with teliospores taken directly from infected *B. macra* inflorescences (Fig. 4).

Preference experiment

A simple laboratory experiment was conducted to determine whether adult beetles might be involved in the dispersal of spores from infected to healthy inflorescences. For this 10 healthy and 10 infected *B. macra* inflorescences were collected from the field, and set upright in water filled flasks so that the shoot remained alive. The 20 stems were placed apart in an insect proof chamber to which 10 adult beetles were

Fig. 4. Scanning electron micrographs comparison of teliospores of *S. amphilophis* found on (A) infected *B. macra* inflorescences and (B) on *Phalacrus* sp.


A
Sporisorium amphilophis
5.00µm
B
Phalacrus sp.
Distance = 7.5µm

released and their daily position on healthy or infected inflorescences for the next 5 days recorded. This experiment was repeated twice.

Length of larval stages

To assess characteristics of the life cycle of the beetle, 20 smut infected inflorescences were collected from the Black Mt. site in March 1993 and examined for beetle eggs. Eggs which were oblong and whitish, were found within the spore mass. Ten inflorescences containing eggs were placed over a circle of filter paper in separate petri dishes. The filter paper was wetted with three drops of water every second day in order to maintain humidity in the petri dish. Larval and pupal development was monitored until adults emerged.

RESULTS

Field observations

(a) *Incidence of adult* Phalacrus *in the field*

Survey 1. *Phalacrus* beetles were only found in the three populations where smut disease was present. In these populations, the levels of smut infection ranged from 19 to 42%. The number of *Phalacrus* beetles was highly correlated ($r^2 = 0.97$) with the proportion of smut infected plants in the populations (Fig. 5).

Survey 2. In general, the abundance of *Phalacrus* sp. was very much lower during the flowering season of 1994-1995 (Fig. 6), than in the previous year. At the beginning of the season (November), when healthy and infected inflorescences of the first *B. macra* plants began to appear, beetles were found in only two populations (Black Mt. and Coppins Crossing). However, by February beetles were found in all five populations. The abundance of beetles in most populations was highest at this time, although at the Uriarra Rd. site the abundance of *Phalacrus* was always very low. There was no apparent relationship between the proportion of infected *B. macra* plants in a population and the number of *Phalacrus* adults (Fig. 7).

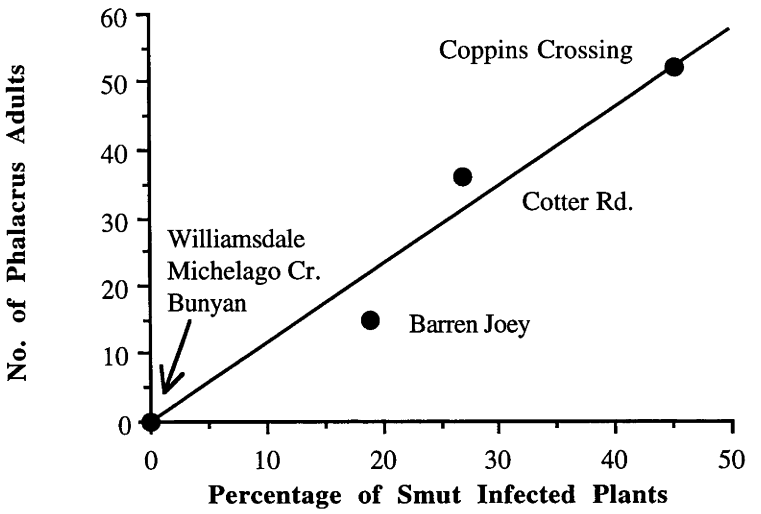


Fig. 5. Relationship between number of *Phalacrus* sp. adults occurring in six *B. macra* populations and the proportion of smut infected plants ($y = -0.58 + 1.17x$, $r^2 = 0.97$).

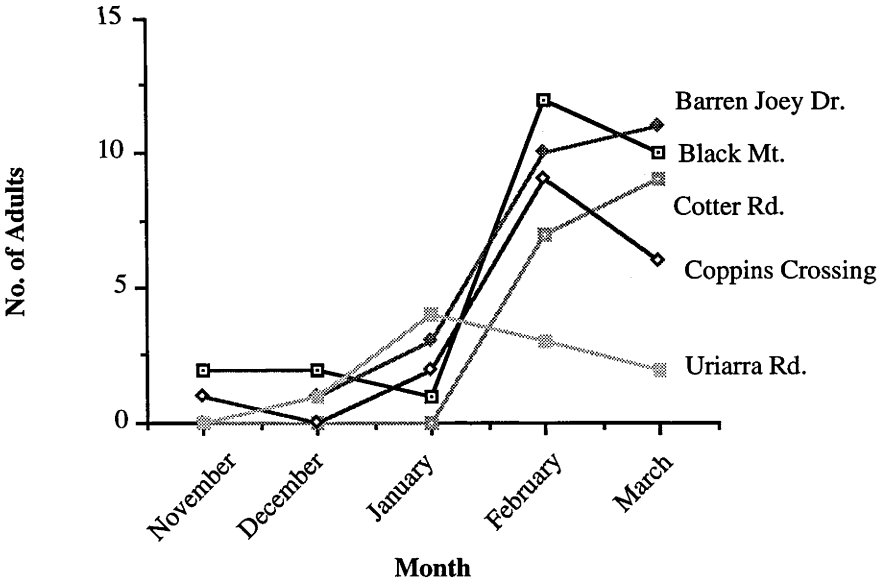


Fig. 6. Number of *Phalacrus* sp. adults caught in five *B. macra* populations during the flowering period November 1994 to March 1995.

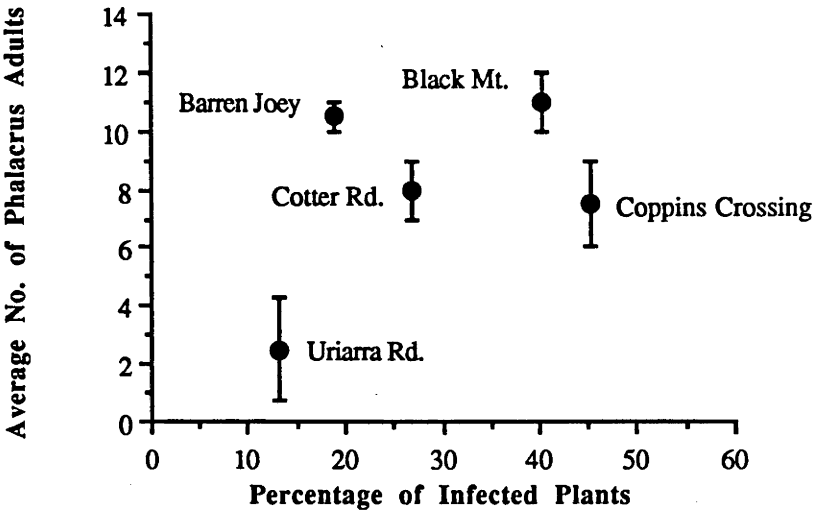


Fig. 7. Comparison of the proportion of smut infected *B. macra* plants and the average number of *Phalacrus* sp. adults found between February and March in five populations. Vertical bars represent ± 1 SE.

(b) *Larval incidence in the field*

The analysis of healthy and infected *B. macra* inflorescences collected in the field showed that only infected inflorescences contained *Phalacrus* larvae. The proportion of smut infected inflorescences with larvae and the number of adults found in each population (survey 1) were highly correlated (Fig. 8).

Most infected heads contained only one larvae. However, in the sample from the Coppins Crossing site, 2 larvae per head were found in three instances (Fig. 9). These results are consistent with a poisson distribution, suggesting random oviposition - larval survival. In addition, the analysis of heads showed that in most cases, larvae were concentrated at the base of the inflorescence; only a few larvae were found in the central part of the head (Fig. 10).

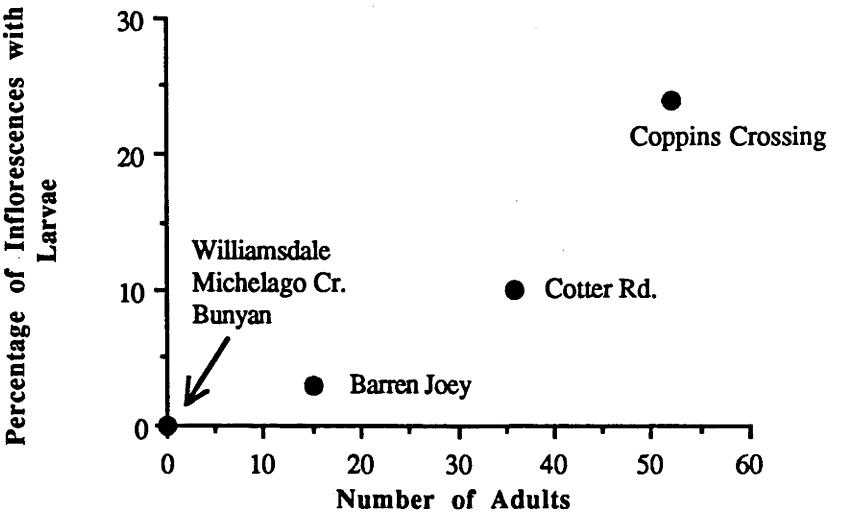


Fig. 8. Percentage of inflorescences with *Phalacrus* sp. larvae and its relation with the number of *Phalacrus* sp. adults in six *B. macra* populations.

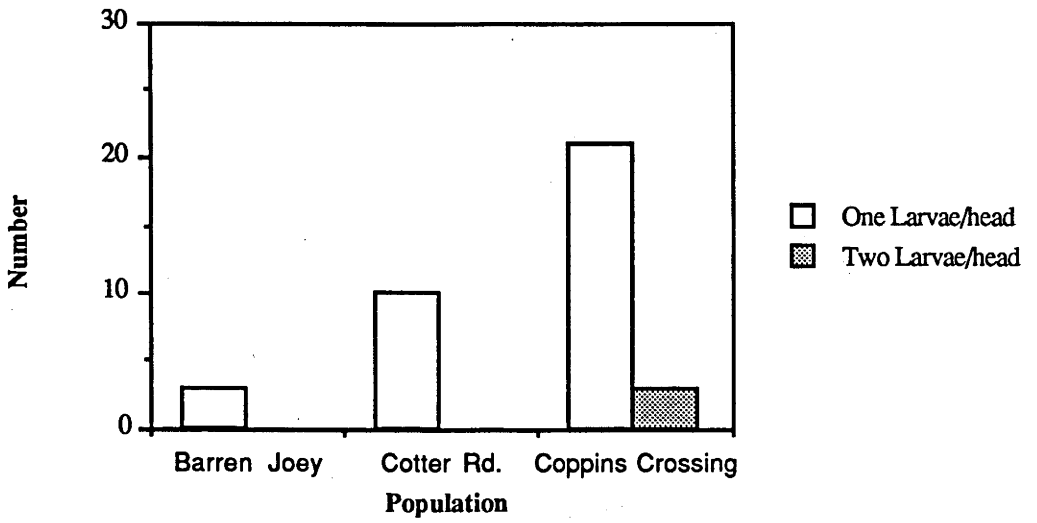


Fig. 9. Number of *Phalacrus* sp. larvae per smutted head in three *B. macra* populations.

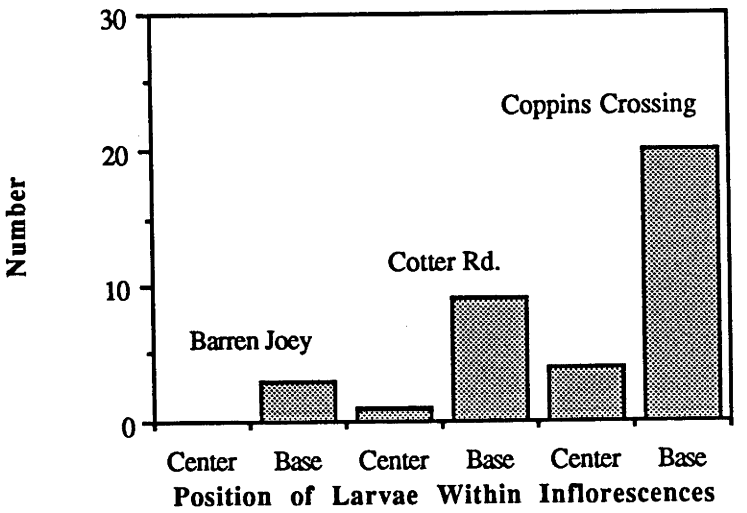


Fig. 10. Number of *Phalacrus* sp. larvae found in different zones within *B. macra* inflorescences from three populations.

(c) *Does* Phalacrus *feed on the smut?*

Scanning electron micrographs of the body of adult *Phalacrus* beetles showed that smut spores could be found on all parts of the body, but principally the legs (Fig. 2). This is shown in Fig. 11 where teliospores of *S. amphilophis* are seen on the mouth parts, abdomen and forewings. Analysis of the gut content of all adults and larvae surveyed showed the presence of large numbers of intact and fragmented teliospores, indicating that both stages of the insect life cycle feed on the spores.

Laboratory observations

Preference experiment

This experiment showed that all beetles kept in the insect chamber for a period of five days, were always found on the infected inflorescences (Table 1). There was no evidence that they visited healthy inflorescences.

Fig. 11. Scanning electron micrographs of different parts of *Phalacrus* sp. body, showing *S. amphilophis* teliospores. (A) Head, (B) mouth parts, (C) forewings and (D) abdomen.

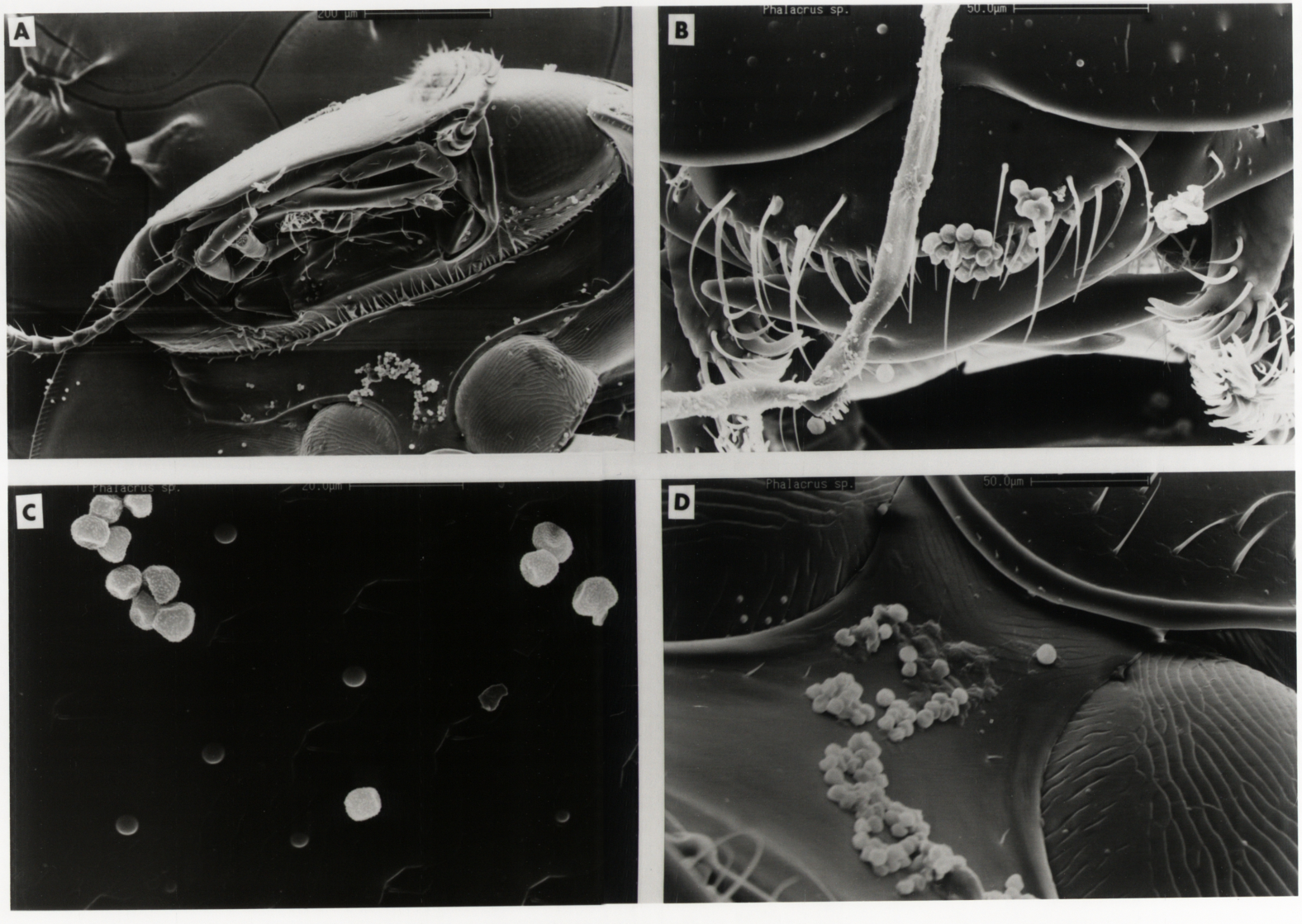

A
200 µm
B
Phalacrus sp.
50.0µm
C
Phalacrus sp.
20.0µm
D
Phalacrus sp.
50.0µm

Table 1. Number of *Phalacrus* sp. adults found on healthy and infected *B. macra* inflorescences for a period of five days.

Day	Number of Beetles	
	Healthy Inflorescences	Infected Inflorescences
1	0	10
2	0	10
3	0	10
4	0	10
5	0	10

Length of larval stages

Eggs were found singly in small clusters of two in the spore mass of infected *B. macra* inflorescences. It was not possible to determine the exact length of the egg period, however larvae emerged 7 to 10 days after inflorescences were collected from the field. Under laboratory conditions, larvae did not remained buried within the spore mass but were found feeding over the surface or on spores on the filter paper. The number of larval instar stages was not determined, but the larval stage lasts from 9 to 12 days. The pupae were very active, gyrating within the pupal chamber, and the pupal period lasted from 13 to 15 days (Table 2).

Table 2. The duration of each stage of the life-cycle of *Phalacrus* sp. estimated under laboratory conditions.

Stage	Average duration [days] ± SE
Egg	8.80 ± 1.40
Larvae	10.20 ± 1.23
Pupae	13.40 ± 1.07

DISCUSSION

The results of the present study have shown that the *Phalacrus* beetle associated with the *B. macra - S. amphilophis* interaction is, like other members of the family Phalacridae, mycophagous. Its spatial and temporal abundance in the field may be determined by the presence of smut infected *B. macra* plants (Fig 5). In 1993 this relationship was clearer and the abundance of adult and larvae was much higher than during the flowering period of 1994-1995. The latter results may well have been influenced by the severe drought conditions which occurred during 1994. In addition, in 1994 the flowering of most healthy and infected *B. macra* plants began later than usual.

The role of Phalacrid insects in the transmission of smut spores from infected to healthy plants is very variable. In some species, for example *P. immarginatus* (associated with sugar cane smut) spores sticking to legs or other body parts have been implicated as source of secondary infections (Agarwal 1956). In such cases, the spread of the infection by these insects seems to be a chance phenomenon and apparently does not involve specific adaptations. In some other associations such as the one involving *P. substriatus* and the smut *Anthracoidea fischeri* in *Carex* spp. insects are important contributors to the transmission of the disease from overwintering sori to new season's inflorescences (Ericson *et al.* 1993). In this particular association the smut is not systemic and adults of *P. substriatus* lay their eggs into florets of *Carex* spp. at the beginning of anthesis, when sori of *A. fischeri* are not present. Larvae of this species of *Phalacrus* feed solely on smut spores, so that the spread of spores by adult beetles is essential for the survival of larvae (Ericson *et al.* 1993). This suggests that some associations with *Phalacrus* spp. are likely to involve specific adaptations.

It is not known whether *Phalacrus* sp. is a vector of *S. amphilophis*. However, the results from the preference experiment as well as the absence of beetles in healthy *B. macra* populations or even from healthy plants in diseased populations, strongly suggests that these insects, even when carrying smut spores sticking to their body, have little role to play in the transport of spores from infected to healthy *B. macra* plants. [Other *Phalacrus* species, such as *P. politus* which feeds on the teliospores of corn also has no apparently role in the spread of disease (Steiner 1984)]. I did not determine if the spores of *S. amphilophis* found in the gut of larvae and adults were

viable, however some studies have shown that spores ingested by some *Phalacrus* larvae are not viable (*P. immarginatus*; Agarwal, 1956), so again spread of the disease in this way might not be common. If these Phalacrid insects have any role to play in the interaction between *S. amphilophis* and *B. macra* it may be one of reducing inoculum production by spore consumption. Whether this "grazing" pressure is sufficiently high as to affect transmission rates is unknown at this stage.

SECTION C

GENERAL DISCUSSION

CHAPTER 7

General Discussion

Fungal plant pathogens have a range of effects on their hosts, from reductions in vigour to the killing of host plants. However, the outcomes of infection are likely to depend not only on the identity of the pathogen, but also on traits related to their life-history strategies. In general, non-systemic pathogens tend to be more aggressive than systemic ones. Their individual effects range from those that kill host plants with considerable rapidity (eg. damping off diseases; Augspurger 1983) to those that individually have little effect (eg. local lesion diseases) but which, given suitable environmental conditions, may increase rapidly to epidemic proportions causing substantial effects on fecundity and longevity (Jarosz and Burdon 1992).

Systemic diseases are also a diverse group of pathogens including organisms that rapidly kill their hosts (vascular wilts) and those that are asymptomatic for much of the time before visibly affecting reproductive output (some rusts and smuts). I exclude here the former category as being a distinct group characterized by internal spread via fragmented mycelium and spores within the xylem of the vascular system. Instead I focus attention on systemic associations in which the pathogen largely grows intercellularly. Among such systemic associations, the effects of the pathogen on the host tend to be more subtle that non-systemic ones, ranging from associations involving reductions in survival of the hosts (eg. the anther-smut *Ustilago violacea* (*Microbotryum violaceum*) infecting *Silene alba*) through to others, such as the systemic rust *Puccinia pratensis* infecting *Pulsatilla pratensis* (Wennström and Ericson 1991), which are closer to symbiotic in nature (Jarosz and Davelos 1995). Again in these associations the outcome of infection is likely to be affected by a range of factors, life-history traits being one of obvious potential importance (Wennström 1994).

Wennström (1994) reviewed some systemic associations and found marked differences in disease expression and host response between groups of plants with different growth patterns (weak and strong lateral growth). His study suggested that the effect of disease on the survival and competitive ability of plants with

weak lateral growth was, in general, less severe than in plants with strong lateral growth. These differences were explained in terms of the consequences for the pathogen of a life style involving perennation within host tissues. In this situation, pathogens having a negative effect on the vigour and survival of the host plant may directly threaten their own survival. Wennström argued that for plants with strong lateral growth the tendency of the host to fragment, would tend to favour rapidly growing, aggressive pathogens (eg. the rust *Puccinia minussensis* infecting *Lactuca sibirica*), because the death of a few infected ramets would not kill the pathogen individual. In contrast, in plants with weak lateral growth such fragmentation does not occur and excessively aggressive systemic pathogens would kill individual hosts and hence themselves. The examples Wennström provided of plants with weak lateral growth all included pathogens with apparently minor effects (eg.the anther-smut *Microbotryum violaceum* infecting *Silene dioica* and the rust *Puccinia pratensis* infecting *Pulsatilla pratensis*).

While Wennström ideas are very interesting, the number of studies used to make these comparisons was very limited and based exclusively on systemic diseases of dicotyledonous plants. Indeed this focus on dicotyledonous plants has tended to cut these studies off from the large and growing body of literature concerning the effects of endophytes on grass hosts (eg. *Epichloe typhina* infecting *Dactylis glomerata; Acremonium coenophialum* infecting *Festuca arundinacea*; Clay 1988). Grasses are an important group of hosts for many systemic fungi, particularly flower smuts, but there have been virtually no studies of smut-grass interactions in natural systems. Monocotyledonous plants are frequently annual or very short-lived perennials (particularly grasses) and like dicotyledonous plants, have a wide range of different growth forms that can be used to increase our understanding of the interplay of hosts growth-form and systemic pathogen spread and persistence. Information available from agricultural systems suggested there might be some important differences between how systemic pathogens interact with grasses and how they interact with dicotyledonous plants.

In response to the current bias of studies of systemic pathogens towards dicotyledonous plants, I have focused my study on monocotyledonous plants, in particular on three associations involving systemic smuts and their grass hosts, which vary in growth form: *Bromus catharticus* and *Bothriochloa macra* are tiller forming species, while *Cynodon dactylon* is a stolon and rhizome forming grass.

The effect of systemic smuts on the competitive ability of hosts

The three host-pathogen systems examined in this thesis showed a range of differential effects some of which undoubtedly reflect their growth form. However, the most striking feature is the consistency of the response of infected plants in terms of their competitive ability. In all three systems, the competitive ability of infected plants was largely unchanged from that of healthy individuals regardless of density. This occurred even though disease markedly reduced growth of infected plants. Only under extreme environmental stress were there signs of an effect of disease in terms of reducing competitive ability. Thus I found that smut reduced the competitive ability of *Bromus catharticus* and *Bothriochloa macra* plants when grown at low nutrient levels, but not under high nutrient conditions. In contrast, the competitive ability of infected *C. dactylon* was not affected by smut, and both healthy and infected individuals competed equally for the same limiting resources, even when grown in poor environments.

These results reflect the implications of casual observations - that smutted grass individuals are quite common and frequently found in swards, lawns, rough pastures and other situations where neighbours are many and close by-situations where competition might be expected to be strong. Certainly, my study of *S. amphilophis* smut on *B. macra* populations showed there was a density effect on smut incidence (Chapter 4). However soils in Australia are skeletal, so that nutrient levels are generally very low, thus placing infected plants under marked environmental stress.

The general lack of effect of smut disease on the competitive ability of infected plants, is an interesting result that differ from that in non-systemic diseases, where infection commonly results in reduced competitive ability of infected plants. For example, Burdon *et al.* (1984) compared the intraspecific competitive abilities of two genotypes of *Chondilla juncea,* one susceptible to the non-systemic rust *Puccinia chondillina* and the other resistant. In the presence of rust the resistant line was a better competitor than the susceptible line, but in the absence of infection, the susceptible plants had a slight competitive advantage. In contrast to the more detailed competition studies carried out with non-systemic associations, the number of studies assessing competitive ability involving systemic interactions is very limited. Most of these studies have claimed positive or negative effects of systemic infections on competitive ability, but only on the basis of reduced biomass (Carlsson and Elmqvist 1992; Wennström and Ericson 1991; Wennström

1994). However as my studies have shown, such data alone fails to directly assess the relative competitive relationships. That is, a reduction in growth does not necessarily imply a reduction in competitive ability.

The effect of growth form on disease expression

A comparison between the expression of disease in the three grass species and other systemic associations involving dicotyledonous plants, suggests that there are many similarities between systems, particularly between those involving hosts with similar morphology. Some of these similarities and differences are shown in Table 1 where aspects of the three host-pathogen systems studied here are compared with representative examples of two dicotyledonous systemic host-pathogen systems, and an endophytic association involving a monocotyledonous plant.

Ustilago bullata and *Sporisorium amphilophis* seem to have very similar effects on their hosts - effects which are also similar to those occurring in the association involving the systemic smut *Microbotryum violaceum* and the dicotyledonous plant *Silene dioica.* (Table 1) These three plant species are characterised by their weak lateral growth. Some studies have shown that smut infection of *Silene dioica* reduces fecundity but only has minimal effects on the survival of infected individuals (Carlsson and Elmqvist 1992). This might suggest that this smut infection has a more benign effect than *U. bullata* on *B. catharticus* or *S. amphilophis* on *B. macra.* However recent studies have shown that the same smut infecting *S. alba* can affect survival of infected plants in hard winters (Thrall and Jarosz 1994). Relatively minor effects on survival are found in other systemic systems involving weak lateral growth. Indeed, in just a system involving infection of *Pulsatilla pratensis* by the systemic rust *Puccinia pratensis*, it has been reported that disease may even enhance the competitive ability of the host (Wennström and Ericson 1991; Wennström 1994).

Cynodon dactylon, on the other hand, responded in a different way to smut infection, with effects that were generally similar to those found in *Lactuca sibirica* infected by the systemic rust *Puccinia minussensis* (Table 1). In both these associations, there was an incomplete transmission of disease within the plant and reduced growth, but competitive ability was differentially affected. While infected *C. dactylon* plants competed equally for the same resources as the healthy individuals, Wennström and Ericson (1992; Wennström 1994) reported reduced

Table 1. Comparison of smut infection effects on *B. catharticus*, *B. macra* and *S. amphilophis* with examples of systemic pathogens and endophytes.

	Pathogen-Host Systems					
	Endophyte	**Systemic pathogens infecting monocotyledonous plants**			**Systemic pathogens infecting dicotyledonous plants**	
Parameter	***Acremonium coenophialum* - *Festuca arundinacea***	***Ustilago bullata* - *Bromus catharticus***	***Sporisorium amphilophis* - *Bothriochloa macra***	***Ustilago cynodontis* - *Cynodon dactylon***	***Microbotryum violaceum* - *Silene dioica* (Smut)**	***Puccinia minussensis* - *Lactuca sibirica* (Rust)**
Reproduction	Stopped	Stopped	Stopped	Stopped	Stopped	Reduced
Host survival	Enhanced	Unaffected	Unaffected	Reduced	Unaffected	Reduced in ramets
Growth	Enhanced	Reduced	Reduced	Reduced	Unaffected	Reduced
Root/shoot ratio	-	Reduced	Reduced	Variable	-	-
Competitive ability	Enhanced	Unaffected	Unaffected	Unaffected	Unaffected *	Reduced *
Disease escape	No	No	No	Yes	No	Yes
Reference	Marks *et al.* 1991	Chapter 2	Chapter 4	Chapter 3	Carlsson and Elmqvist 1992	Wennström and Ericson 1992

* Competitive effects were inferred on a basis of yield changes.

competitive ability on infected *L. sibirica* plants. However, their claim of reduced competitive ability was based on the implicit assumption that reduced growth is equated with reduced competitive ability. My studies have shown, that this is not necessarily the case.

It has been suggested that systemic fungi may be important selective agents affecting growth patterns of clonal plants. So that some systemic pathogens may favour lateral growth or fragmentation of ramets, resulting in the escape of host plants from disease. On the other hand, it has been suggested that mutualistic associations, in particular those involving endophytic fungi may favour slow growth and delayed fragmentation so that spread of the endophyte within the host tissues is enhanced (Wennström and Ericson 1992). Despite the total castration of infected plants, infection by these fungi commonly results in enhanced survival, growth and competitive ability.

Survival in the field

The severity of disease in any population, and hence its evolutionary consequences, is determined by the combined effects of aspects of the abiotic and biotic environment. Hence, growing conditions can affect the outcome of infection by systemic pathogens and the survival of plants in the field.

Smut infection in the three host-pathogen associations presented here, resulted in the complete sterility of their hosts, reducing fitness to zero unless the plant could escape infection. Infected plants still impact on their neighbours in populations. Reductions in their growth are likely to reduce the ability of infected plants to respond to stressful field conditions. In the interactions studied, the negative effects of infection were often subtle. Thus while dry matter production was always reduced, in a benign environment neither competitive ability nor survival were affected. However the effects of smut infection on these parameters were highly dependent on the environmental conditions where the plants grew, and under more stressful conditions both survival and competitive ability was reduced. However, it appears that the fungus has successfully taken over the plant to its own ends as a host for fungal; spore production.

Extrapolation of glasshouse-derived results to the field always has some dangers. However, the complementarity of my glasshouse competition studies of healthy and infected *B. macra* and field observations, strongly suggests that such extrapolation is reasonable. Thus the incidence of smut-infected plants in the core areas of individual *B. macra* populations was lower than that at the edge. Given

the very poor soils on which the populations typically grew, competition in these relatively undisturbed core areas would tend to suppress infected individuals. This coupled with their poor root-shoot ratio would make such plants potentially vulnerable to other episodic stresses in the environment like drought or frosting. The subtleness of these effects has recently been shown in *Silene alba* infected with *Ustilago violacea* (*Microbotryum violaceum*) where marked differences in the field performance (survival) of healthy and infected individuals was only seen after severe winter conditions (Thrall and Jarosz 1994). A similar interaction seems to be the most plausible reason for the changing patterns of incidence of S. *amphilophis* infection I recorded in my field survey of *B. macra* populations.

A major difference between smuts infecting grasses and dicotyledonous plants is the general tendency of the former to infect their hosts at the seedling germination/ establishment phase while for dicotyledonous plants infection of adults is more important. During the early phases of the host-life cycle, infection by seed-infecting systemic-smuts, has variable effects on the germination and seedling establishment of their hosts. However seedlings-infecting smuts tend to have little effect on germination of their hosts. For example, germination of *Bromus willdenowii* seeds infected by *Ustilago bullata* is very similar to that of healthy seeds (Luttrell and Craigmiles 1961). Similarly, my studies showed that *U. bullata* and *U. cynodontis* had little effect on the germination of their host grasses (Chapter 2 and 3) although in the former case seedling growth was noticeably reduced. At higher densities than used here this might have reduced survival in a competitive situation. Overall, however, these effects suggest that at early stages of the life-cycle, evolution may favour attributes that minimise damage to host plants, so that the survival of the smut pathogen is assured. Interactions in which seedling growth is severely depressed by infection would appear to be evolutionary unstable. This unaffected germination of *B. catharticus* and *C. dactylon* seeds, may explain the high incidence of smutted plants found in field conditions. However, as I have shown from my competition experiments infected plants do 'pay' a penalty in total growth, changed root/shoot ratio and sometimes reduced competitive ability. Furthermore, survival of adults after flowering may well be reduced. Here I did not address the question directly. However, Falloon (1976) reported increased mortality of *Bromus catharticus* infected by *U. bullata* after flowering.

Selection directions along the *r-K* continuum

The effects of systemic pathogens or endophytes on the competitive ability of their hosts appear to form a continuum, that ranges from reductions in the competitive ability of infected plants such as the systemic smut *Urocystis trientalis* infecting *Trientalis europaea* (Wennström and Ericson 1990); toward those fungi having no apparent effect (eg. the systemic smut *Microbotryum violaceum* infecting *Silene dioica*; Carlsson and Elmqvist 1992) to those enhancing the competitive abilities of infected individuals (eg. the endophyte *Acremonium coenophialum* infecting *Festuca arundinacea*; Marks *et al.* 1991). As I have pointed out (Chapter 2) the different forms of parasitism found in fungal associations may be seen as a continuum that ranges from extreme ***r*** type pathogens characterised by high fecundity and short life-cycles to ***K*** type pathogens characterised by lower fecundity, longer life-cycles and intimate associations with their hosts; and finally to the ultimate expression of mutualistic associations developed by endophytic fungi.

The extreme ***r*** end of this continuum is characterized by non-systemic discrete lesion diseases such as *Puccinia chondrillina* attacking *Chondrilla juncea* and *Puccinia lagenophorae* attacking *Senecio vulgaris.* These reduce the reproduction, survival and competitive ability of their hosts (Burdon *et al.* 1981; Paul and Ayres 1986b, 1987a). Pathogens involved in such associations typically have short generation times and high fecundity per generation, and selection for greater fitness of the pathogen individual may favour increasing aggressiveness even though the cumulative effect of very many lesions may result in death of the host plant.

In contrast, systemic pathogens have longer generation times and survive from year to year within infected plants. For this type of pathogen increasing aggressiveness is not likely to be favoured as this could kill the host [and hence the pathogen] before reproduction. As a consequence, the systemic associations studied here, appear to combine selective elements from both ends of the ***r-K*** continuum of parasitism. The low effect of the pathogens on survival and competitive ability of infected plants are characteristics that suggest a close relationship, where selective forces have apparently favoured low aggressivity for the three smut species, placing these associations closer to the ***K*** end. In contrast, the greatly reduced host-size, the general reduction in root-shoot ratios, the high production of spores and the total castration of the host grasses, all indicate that

these associations are still harmful for the host plants. Moreover, these associations are clearly still far more pathogenic than endophytic associations like *Acremonium coenophialum-Festuca arundinacea* (Table 1) in which host survival, growth and competitive ability is enhanced by infection (Marks *et al.* 1991).

The overwhelming diversity of plant pathogens and the patchy nature of the data available makes it difficult to generalise about their ecological interactions. The results of my study have shown that the outcome of infection by pathogens is not only determined by the identity of the pathogen but by many different traits such as the life histories of both pathogens and hosts. In this light it is not surprising that in some systems involving systemic diseases it has been hard to detect any effect on host fitness. To increase the understanding of disease dynamics, more studies involving different pathogens and hosts must be carried out. In addition, future work should study the interaction of the effects of different biotic and abiotic factors on the outcome of infection as well as the effects of infection on different stages of the host life cycle.

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